(54) Title: LCES AS MODIFIERS OF THE P53 PATHWAY AND METHODS OF USE

(57) Abstract: Human LCE genes are identified as modulators of the p53 pathway, and thus are therapeutic targets for disorders associated with defective p53 function. Methods for identifying modulators of p53, comprising screening for agents that modulate the activity of LCE are provided.

LCEs AS MODIFIERS OF THE p53 PATHWAY AND METHODS OF USE

REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional patent applications 60/296,076

5   filed 6/5/2001, 60/328,605 filed 10/10/2001, 60/357,253 filed 2/15/2002, and 60/361,196

filed 3/1/2002. The contents of the prior applications are hereby incorporated in their

entirety.

BACKGROUND OF THE INVENTION

10   The p53 gene is mutated in over 50 different types of human cancers, including

familial and spontaneous cancers, and is believed to be the most commonly mutated gene

in human cancer (Zambetti and Levine, FASEB (1993) 7:855-865; Hollstein, et al.,

Nucleic Acids Res. (1994) 22:3551-3555). Greater than 90% of mutations in the p53 gene

are missense mutations that alter a single amino acid that inactivates p53 function.

15   Aberrant forms of human p53 are associated with poor prognosis, more aggressive tumors,

metastasis, and short survival rates (Mitsudomi et al., Clin Cancer Res 2000 Oct;

6(10):4055-63; Koshland, Science (1993) 262:1953).

The human p53 protein normally functions as a central integrator of signals including

DNA damage, hypoxia, nucleotide deprivation, and oncogene activation (Prives, Cell

20   (1998) 95:5-8). In response to these signals, p53 protein levels are greatly increased with

the result that the accumulated p53 activates cell cycle arrest or apoptosis depending on

the nature and strength of these signals. Indeed, multiple lines of experimental evidence

have pointed to a key role for p53 as a tumor suppressor (Levine, Cell (1997) 88:323-331).

For example, homozygous p53 "knockout" mice are developmentally normal but exhibit

25   nearly 100% incidence of neoplasia in the first year of life (Donehower et al., Nature

(1992) 356:215-221).

The biochemical mechanisms and pathways through which p53 functions in normal

and cancerous cells are not fully understood, but one clearly important aspect of p53

function is its activity as a gene-specific transcriptional activator. Among the genes with

30   known p53-response elements are several with well-characterized roles in either regulation

of the cell cycle or apoptosis, including GADD45, p21/Waf1/Cip1, cyclin G, Bax, IGF-

BP3, and MDM2 (Levine, Cell (1997) 88:323-331).

Several essential organs (e.g., lungs, kidney, lymphatic system and vasculature) are

made up of complex networks of tube-like structures that serve to transport and exchange

fluids, gases, nutrients and waste. The formation of these complex branched networks occurs by the evolutionarily conserved process of branching morphogenesis, in which successive ramification occurs by sprouting, pruning and remodeling of the network. During human embryogenesis, blood vessels develop via two processes: vasculogenesis, whereby endothelial cells are born from progenitor cell types; and angiogenesis, in which new capillaries sprout from existing vessels.

Branching morphogenesis encompasses many cellular processes, including proliferation, survival/apoptosis, migration, invasion, adhesion, aggregation and matrix remodeling. Numerous cell types contribute to branching morphogenesis, including endothelial, epithelial and smooth muscle cells, and monocytes. Gene pathways that modulate the branching process function both within the branching tissues as well as in other cells, e.g., certain monocytes can promote an angiogenic response even though they may not directly participate in the formation of the branch structures.

An increased level of angiogenesis is central to several human disease pathologies, including rheumatoid arthritis and diabetic retinopathy, and, significantly, to the growth, maintenance and metastasis of solid tumors (for detailed reviews see Liotta LA et al, 1991, Cell 64:327-336; Folkman J., 1995 Nature Medicine 1:27-31; Hanahan D and Folkman J, 1996 Cell 86:353-364). Impaired angiogenesis figures prominently in other human diseases, including heart disease, stroke, infertility, ulcers and scleroderma.

The transition from dormant to active blood vessel formation involves modulating the balance between angiogenic stimulators and inhibitors. Under certain pathological circumstances an imbalance arises between local inhibitory controls and angiogenic inducers resulting in excessive angiogenesis, while under other pathological conditions an imbalance leads to insufficient angiogenesis. This delicate equilibrium of pro- and anti-angiogenic factors is regulated by a complex interaction between the extracellular matrix, endothelial cells, smooth muscle cells, and various other cell types, as well as environmental factors such as oxygen demand within tissues.

Most known angiogenesis genes, their biochemical activities, and their organization into signaling pathways are employed in a similar fashion during angiogenesis in human, mouse and Zebrafish, as well as during branching morphogenesis of the *Drosophila* trachea. Accordingly, *Drosophila* tracheal development and zebrafish vascular development provide useful models for studying mammalian angiogenesis (Metzger RJ, Krasnow MA. Science. 1999. 284:1635-9; Roman BL, and Weinstein BM. Bioessays 2000, 22:882-93).

In mammals, most of the fatty acids that are synthesized de novo possess chain lengths of 16-18 carbons. These long chain fatty acids constitute more than 90% of all fatty acids present in cells. They are important components of membranes, and they represent the largest energy storage reservoir in animals. The highest rate of de novo fatty acid

5   synthesis occurs in liver, which converts excess glucose into fatty acids for storage and transport. Glycolysis converts glucose to pyruvate, which is converted to citrate in the mitochondria and transported to the cytosol. Cytosolic ATP citrate lyase generates acetyl-CoA, the precursor of fatty acids and cholesterol. Acetyl-CoA is carboxylated by acetyl-CoA carboxylase (ACC) to form malonyl-CoA. The multifunction enzyme fatty acid

10   synthase (FAS) uses malonyl-CoA, acetyl-CoA, and NADPH to elongate fatty acids in 2-carbon increments. The principal end product of FAS in rodents is palmitic acid, which contains 16 carbons and is designated 16:0. A high proportion of this palmitic acid is then converted to stearate.

At a molecular level, fatty acid elongases have been characterized most extensively by

15   genetic studies in yeast. Yeast ELO1 elongates C14 to C16 fatty acids and is designated a long chain fatty acid elongase (Toke DA et al, 1996, J Biol Chem 271:18413-18422). The ELO2 and ELO3 genes encode very long chain elongases that produce fatty acids of 24 to 26 carbons (Oh CS et al, 1997, J Biol Chem 272:17376-17384). The mouse gene, Cig30, encodes the mouse version of ELO2, and Ssc1 encodes the ortholog of ELO3, both of

20   which are very long chain elongases (Tvrdik P et al., 2000, J Cell Biol 149:707-718). CIG30 and other Fatty acid elongases have been implicated in sphingolipid biosynthesis. In certain cells, sphingosine kinase is exported to extracellular space, where it phosphorylates sphingosine to make phospho-sphingosine (S1P) (Hla et al., Science 2001, 294:1875-8). S1P is a potent second messenger that binds to the EDG1 GPCR to mediate

25   migration, survival, morphogenesis and proliferation. S1P and EDG1 have been shown to be involved in endothelial cell migration and vascular maturation in vivo (Ancellin et al., J Biol Chem 2002, 277:6667-75).

A murine long chain fatty acyl elongase (LCE) that shares sequence identity with previously identified very long chain fatty acid elongases has been identified. LCE

30   mRNA is highly expressed in liver and adipose tissue and is thought to catalyze the rate limiting condensing step in addition of 2-carbon units to C12-C16 fatty acids (Moon YA et al., 2001, J Biol Chem 276:45358-66). One of the human LCE genes, ELOVL4, has been identified as a disease gene –Autosomal Dominant Stargardt-like Macular Dystrophy (STGD3) – associated with inherited forms of macular degeneration characterized by

decreased visual acuity, macular atrophy and extensive fundus flecks (Zhang et al., Nat Genet 2001, 27:89-93). All afflicted members in five related families carry a 5 bp deletion (the so-called 797-801 del AACTT) of the gene.

The ability to manipulate the genomes of model organisms such as *Drosophila*

5   provides a powerful means to analyze biochemical processes that, due to significant evolutionary conservation, have direct relevance to more complex vertebrate organisms. Due to a high level of gene and pathway conservation, the strong similarity of cellular processes, and the functional conservation of genes between these model organisms and mammals, identification of the involvement of novel genes in particular pathways and

10   their functions in such model organisms can directly contribute to the understanding of the correlative pathways and methods of modulating them in mammals (see, for example, Mechler BM et al., 1985 EMBO J 4:1551-1557; Gateff E. 1982 Adv. Cancer Res. 37: 33-74; Watson KL., et al., 1994 J Cell Sci. 18: 19-33; Miklos GL, and Rubin GM. 1996 Cell 86:521-529; Wassarman DA, et al., 1995 Curr Opin Gen Dev 5: 44-50; and Booth DR.

15   1999 Cancer Metastasis Rev. 18: 261-284). For example, a genetic screen can be carried out in an invertebrate model organism having underexpression (e.g. knockout) or overexpression of a gene (referred to as a "genetic entry point") that yields a visible phenotype. Additional genes are mutated in a random or targeted manner. When a gene mutation changes the original phenotype caused by the mutation in the genetic entry point,

20   the gene is identified as a "modifier" involved in the same or overlapping pathway as the genetic entry point. When the genetic entry point is an ortholog of a human gene implicated in a disease pathway, such as p53, modifier genes can be identified that may be attractive candidate targets for novel therapeutics.

All references cited herein, including sequence information in referenced Genbank

25   identifier numbers and website references, are incorporated herein in their entireties.

SUMMARY OF THE INVENTION

We have discovered genes that modify the p53 pathway in *Drosophila*, and identified their human orthologs, hereinafter referred to as LCEs. The invention provides methods

30   for utilizing these p53 modifier genes and polypeptides to identify candidate therapeutic agents that can be used in the treatment of disorders associated with defective p53 function. Preferred LCE-modulating agents specifically bind to LCE polypeptides and restore p53 function. Other preferred LCE-modulating agents are nucleic acid modulators such as antisense oligomers and RNAi that repress LCE gene expression or product

activity by, for example, binding to and inhibiting the respective nucleic acid (i.e. DNA or mRNA).

LCE-specific modulating agents may be evaluated by any convenient *in vitro* or *in vivo* assay for molecular interaction with an LCE polypeptide or nucleic acid. In one

5   embodiment, candidate p53 modulating agents are tested with an assay system comprising a LCE polypeptide or nucleic acid. Candidate agents that produce a change in the activity of the assay system relative to controls are identified as candidate p53 modulating agents. The assay system may be cell-based or cell-free. LCE-modulating agents include LCE related proteins (e.g. dominant negative mutants, and biotherapeutics); LCE-specific

10   antibodies; LCE-specific antisense oligomers and other nucleic acid modulators; and chemical agents that specifically bind LCE or compete with LCE binding target. In one specific embodiment, a small molecule modulator is identified using a binding assay. In specific embodiments, the screening assay system is selected from an apoptosis assay, a cell proliferation assay, an angiogenesis assay, and a hypoxic induction assay.

15   In another embodiment, candidate p53 pathway modulating agents are further tested using a second assay system that detects changes in the p53 pathway, such as angiogenic, apoptotic, or cell proliferation changes produced by the originally identified candidate agent or an agent derived from the original agent. The second assay system may use cultured cells or non-human animals. In specific embodiments, the secondary assay

20   system uses non-human animals, including animals predetermined to have a disease or disorder implicating the p53 pathway, such as an angiogenic, apoptotic, or cell proliferation disorder (e.g. cancer).

The invention further provides methods for modulating the p53 pathway in a mammalian cell by contacting the mammalian cell with an agent that specifically binds a

25   LCE polypeptide or nucleic acid. The agent may be a small molecule modulator, a nucleic acid modulator, or an antibody and may be administered to a mammalian animal predetermined to have a pathology associated the p53 pathway.

The invention further provides methods of identifying candidate branching morphogenesis modulating agents and methods of modulating branching morphogenesis

30   in mammalian cells using an LCE.

DETAILED DESCRIPTION OF THE INVENTION

Genetic screens were designed to identify modifiers of the p53 pathway in *Drosophila* in which p53 was overexpressed in the wing (Ollmann M, et al., Cell 2000 101: 91-101).

The baldspot gene was identified as a modifier of the p53 pathway. Accordingly, vertebrate orthologs of these modifiers, and preferably the human orthologs, LCE genes (i.e., nucleic acids and polypeptides) are attractive drug targets for the treatment of pathologies associated with a defective p53 signaling pathway, such as cancer.

5　　　　We have further identified an LCE (ELOVL4) that is involved in branching morphogenesis, specifically angiogenesis and vasculogenesis, as further described in the Examples. Accordingly, ELOVL4 is an attractive drug target for the treatment of pathologies related to branching morphogenesis, including the treatment of tumors whose growth is associated with increased angiogenesis.

10　　　In vitro and in vivo methods of assessing LCE function are provided herein. Modulation of the LCE or their respective binding partners is useful for understanding the association of the p53 pathway and its members in normal and disease conditions and for developing diagnostics and therapeutic modalities for p53 related pathologies. Modulation of the ELOVL4 or its binding partners is further useful for elucidating the process of

15　　　branching morphogenesis and the association of branching morphogenesis with the p53 pathway, and for developing diagnostic and therapeutic modalities for pathologies associated with the branching morphogenesis. As used herein, branching morphogenesis encompasses the numerous cellular process involved in the formation of branched networks, including proliferation, survival/apoptosis, migration, invasion, adhesion,

20　　　aggregation and matrix remodeling. As used herein, pathologies associated with branching morphogenesis encompass pathologies where branching morphogenesis contributes to maintaining the healthy state, as well as pathologies whose course may be altered by modulation of the branching morphogenesis.

　　　　　LCE-modulating agents that act by inhibiting or enhancing LCE expression, directly or

25　　　indirectly, for example, by affecting an LCE function such as enzymatic (e.g., catalytic) or binding activity, can be identified using methods provided herein. LCE modulating agents are useful in diagnosis, therapy and pharmaceutical development.

Nucleic acids and polypeptides of the invention

30　　　Sequences related to LCE nucleic acids and polypeptides that can be used in the invention are disclosed in Genbank (referenced by Genbank identifier (GI) number) as GI#s 10444344 (SEQ ID NO:1), 13129087 (SEQ ID NO:3), 10440044 (SEQ ID NO:4), 12044042 (SEQ ID NO:5), 12232378 (SEQ ID NO:6), and 18576451 (SEQ ID NO:8) for nucleic acid, and GI#s 10444345 (SEQ ID NO:9), 13129088 (SEQ ID NO:11), 10440045

(SEQ ID NO:12), 12044043 (SEQ ID NO:13), 12232379 (SEQ ID NO:14), and 17454617 (SEQ ID NO:16) for polypeptides. Additionally, nucleic acid sequences of SEQ ID NOs: 2 and 7, and polypeptide sequences of SEQ ID NOs: 10 and 15 can also be used in the invention.

5   LCEs are fatty acid elongase proteins with GNS1/SUR4 domains. The term "LCE polypeptide" refers to a full-length LCE protein or a functionally active fragment or derivative thereof. A "functionally active" LCE fragment or derivative exhibits one or more functional activities associated with a full-length, wild-type LCE protein, such as antigenic or immunogenic activity, enzymatic activity, ability to bind natural cellular

10   substrates, etc. The functional activity of LCE proteins, derivatives and fragments can be assayed by various methods known to one skilled in the art (Current Protocols in Protein Science (1998) Coligan *et al.*, eds., John Wiley & Sons, Inc., Somerset, New Jersey) and as further discussed below. For purposes herein, functionally active fragments also include those fragments that comprise one or more structural domains of an LCE, such as

15   a binding domain. Protein domains can be identified using the PFAM program (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2; http://pfam.wustl.edu). For example, the GNS1/SUR4 domain (PFAM 01151) of LCE from GI#s 10444345, 13129088, 12232379, and 17454617 (SEQ ID NOs:9, 11, 14, and 16, respectively) are located respectively at approximately amino acid residues 1-235, 10 to 265, 9 to 289, and 55 to 270. Methods for

20   obtaining LCE polypeptides are also further described below. In some embodiments, preferred fragments are functionally active, domain-containing fragments comprising at least 25 contiguous amino acids, preferably at least 50, more preferably 75, and most preferably at least 100 contiguous amino acids of any one of SEQ ID NOs:9, 10, 11, 12, 13, 14, 15, or 16 (an LCE). In further preferred embodiments, the fragment comprises the

25   entire GNS1/SUR4 (functionally active) domain.
          The term "LCE nucleic acid" refers to a DNA or RNA molecule that encodes a LCE polypeptide. Preferably, the LCE polypeptide or nucleic acid or fragment thereof is from a human, but can also be an ortholog, or derivative thereof with at least 70% sequence identity, preferably at least 80%, more preferably 85%, still more preferably 90%, and

30   most preferably at least 95% sequence identity with LCE. Normally, orthologs in different species retain the same function, due to presence of one or more protein motifs and/or 3-dimensional structures. Orthologs are generally identified by sequence homology analysis, such as BLAST analysis, usually using protein bait sequences. Sequences are assigned as a potential ortholog if the best hit sequence from the forward BLAST result

retrieves the original query sequence in the reverse BLAST (Huynen MA and Bork P, Proc Natl Acad Sci (1998) 95:5849-5856; Huynen MA *et al.*, Genome Research (2000) 10:1204-1210). Programs for multiple sequence alignment, such as CLUSTAL (Thompson JD et al, 1994, Nucleic Acids Res 22:4673-4680) may be used to highlight

5   conserved regions and/or residues of orthologous proteins and to generate phylogenetic trees. In a phylogenetic tree representing multiple homologous sequences from diverse species (e.g., retrieved through BLAST analysis), orthologous sequences from two species generally appear closest on the tree with respect to all other sequences from these two species. Structural threading or other analysis of protein folding (e.g., using software by

10   ProCeryon, Biosciences, Salzburg, Austria) may also identify potential orthologs. In evolution, when a gene duplication event follows speciation, a single gene in one species, such as *Drosophila*, may correspond to multiple genes (paralogs) in another, such as human. As used herein, the term "orthologs" encompasses paralogs. As used herein, "percent (%) sequence identity" with respect to a subject sequence, or a specified portion

15   of a subject sequence, is defined as the percentage of nucleotides or amino acids in the candidate derivative sequence identical with the nucleotides or amino acids in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul *et al.*, J. Mol. Biol. (1997) 215:403-410;

20   http://blast.wustl.edu/blast/README.html) with all the search parameters set to default values. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. A % identity value is determined by the number of matching identical nucleotides or amino

25   acids divided by the sequence length for which the percent identity is being reported. "Percent (%) amino acid sequence similarity" is determined by doing the same calculation as for determining % amino acid sequence identity, but including conservative amino acid substitutions in addition to identical amino acids in the computation.

A conservative amino acid substitution is one in which an amino acid is substituted for

30   another amino acid having similar properties such that the folding or activity of the protein is not significantly affected. Aromatic amino acids that can be substituted for each other are phenylalanine, tryptophan, and tyrosine; interchangeable hydrophobic amino acids are leucine, isoleucine, methionine, and valine; interchangeable polar amino acids are glutamine and asparagine; interchangeable basic amino acids are arginine, lysine and

histidine; interchangeable acidic amino acids are aspartic acid and glutamic acid; and interchangeable small amino acids are alanine, serine, threonine, cysteine and glycine.

Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, Advances in Applied Mathematics 2:482-489; database: European Bioinformatics Institute http://www.ebi.ac.uk/MPsrch/; Smith and Waterman, 1981, J. of Molec.Biol., 147:195-197; Nicholas et al., 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" (www.psc.edu) and references cited therein.; W.R. Pearson, 1991, Genomics 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff (Dayhoff: Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 Nucl. Acids Res. 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."

Derivative nucleic acid molecules of the subject nucleic acid molecules include sequences that hybridize to the nucleic acid sequence of any of SEQ ID NOs:1, 2, 3, 4, 5, 6 7, or 8. The stringency of hybridization can be controlled by temperature, ionic strength, pH, and the presence of denaturing agents such as formamide during hybridization and washing. Conditions routinely used are set out in readily available procedure texts (*e.g.*, Current Protocol in Molecular Biology, Vol. 1, Chap. 2.10, John Wiley & Sons, Publishers (1994); Sambrook *et al.*, Molecular Cloning, Cold Spring Harbor (1989)). In some embodiments, a nucleic acid molecule of the invention is capable of hybridizing to a nucleic acid molecule containing the nucleotide sequence of any one of SEQ ID NOs:1, 2, 3, 4, 5, 6, 7, or 8 under stringent hybridization conditions that comprise: prehybridization of filters containing nucleic acid for 8 hours to overnight at 65° C in a solution comprising 6X single strength citrate (SSC) (1X SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5X Denhardt's solution, 0.05% sodium pyrophosphate and 100 μg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C in a solution containing 6X SSC, 1X Denhardt's solution, 100 μg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C for 1h in a solution containing 0.2X SSC and 0.1% SDS (sodium dodecyl sulfate).

In other embodiments, moderately stringent hybridization conditions are used that comprise: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.1% PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA; hybridization for
5   18-20h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C in a solution containing 2X SSC and 0.1% SDS.

Alternatively, low stringency conditions can be used that comprise: incubation for 8
10   hours to overnight at 37° C in a solution comprising 20% formamide, 5 x SSC, 50 mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured sheared salmon sperm DNA; hybridization in the same buffer for 18 to 20 hours; and washing of filters in 1 x SSC at about 37° C for 1 hour.

15   **Isolation, Production, Expression, and Mis-expression of LCE Nucleic Acids and Polypeptides**

LCE nucleic acids and polypeptides, useful for identifying and testing agents that modulate LCE function and for other applications related to the involvement of LCE in the p53 pathway. LCE nucleic acids and derivatives and orthologs thereof may be obtained
20   using any available method. For instance, techniques for isolating cDNA or genomic DNA sequences of interest by screening DNA libraries or by using polymerase chain reaction (PCR) are well known in the art. In general, the particular use for the protein will dictate the particulars of expression, production, and purification methods. For instance, production of proteins for use in screening for modulating agents may require methods
25   that preserve specific biological activities of these proteins, whereas production of proteins for antibody generation may require structural integrity of particular epitopes. Expression of proteins to be purified for screening or antibody production may require the addition of specific tags (*e.g.*, generation of fusion proteins). Overexpression of an LCE protein for assays used to assess LCE function, such as involvement in cell cycle regulation or
30   hypoxic response, may require expression in eukaryotic cell lines capable of these cellular activities. Techniques for the expression, production, and purification of proteins are well known in the art; any suitable means therefore may be used (e.g., Higgins SJ and Hames BD (eds.) Protein Expression: A Practical Approach, Oxford University Press Inc., New York 1999; Stanbury PF et al., Principles of Fermentation Technology, 2nd edition,

Elsevier Science, New York, 1995; Doonan S (ed.) Protein Purification Protocols, Humana Press, New Jersey, 1996; Coligan JE et al, Current Protocols in Protein Science (eds.), 1999, John Wiley & Sons, New York). In particular embodiments, recombinant LCE is expressed in a cell line known to have defective p53 function (e.g. SAOS-2

5   osteoblasts, H1299 lung cancer cells, C33A and HT3 cervical cancer cells, HT-29 and DLD-1 colon cancer cells, among others, available from American Type Culture Collection (ATCC), Manassas, VA). The recombinant cells are used in cell-based screening assay systems of the invention, as described further below.

The nucleotide sequence encoding an LCE polypeptide can be inserted into any

10   appropriate expression vector. The necessary transcriptional and translational signals, including promoter/enhancer element, can derive from the native LCE gene and/or its flanking regions or can be heterologous. A variety of host-vector expression systems may be utilized, such as mammalian cell systems infected with virus (*e.g.* vaccinia virus, adenovirus, *etc.*); insect cell systems infected with virus (*e.g.* baculovirus);

15   microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, plasmid, or cosmid DNA. A host cell strain that modulates the expression of, modifies, and/or specifically processes the gene product may be used.

To detect expression of the LCE gene product, the expression vector can comprise a promoter operably linked to an LCE gene nucleic acid, one or more origins of replication,

20   and, one or more selectable markers (*e.g.* thymidine kinase activity, resistance to antibiotics, *etc.*). Alternatively, recombinant expression vectors can be identified by assaying for the expression of the LCE gene product based on the physical or functional properties of the LCE protein in *in vitro* assay systems (*e.g.* immunoassays).

The LCE protein, fragment, or derivative may be optionally expressed as a fusion, or

25   chimeric protein product (i.e. it is joined via a peptide bond to a heterologous protein sequence of a different protein), for example to facilitate purification or detection. A chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other using standard methods and expressing the chimeric product. A chimeric product may also be made by protein synthetic techniques,

30   *e.g.* by use of a peptide synthesizer (Hunkapiller *et al.*, Nature (1984) 310:105-111).

Once a recombinant cell that expresses the LCE gene sequence is identified, the gene product can be isolated and purified using standard methods (*e.g.* ion exchange, affinity, and gel exclusion chromatography; centrifugation; differential solubility; electrophoresis). Alternatively, native LCE proteins can be purified from natural sources, by standard

methods (*e.g.* immunoaffinity purification). Once a protein is obtained, it may be quantified and its activity measured by appropriate methods, such as immunoassay, bioassay, or other measurements of physical properties, such as crystallography.

The methods of this invention may also use cells that have been engineered for altered

5   expression (mis-expression) of LCE or other genes associated with the p53 pathway. As used herein, mis-expression encompasses ectopic expression, over-expression, under-expression, and non-expression (*e.g.* by gene knock-out or blocking expression that would otherwise normally occur).

10   **Genetically modified animals**

Animal models that have been genetically modified to alter LCE expression may be used in *in vivo* assays to test for activity of a candidate p53 modulating agent, or to further assess the role of LCE in a p53 pathway process such as apoptosis or cell proliferation. Preferably, the altered LCE expression results in a detectable phenotype, such as

15   decreased or increased levels of cell proliferation, angiogenesis, or apoptosis compared to control animals having normal LCE expression. The genetically modified animal may additionally have altered p53 expression (e.g. p53 knockout). Preferred genetically modified animals are mammals such as primates, rodents (preferably mice), cows, horses, goats, sheep, pigs, dogs and cats. Preferred non-mammalian species include zebrafish, *C.*

20   *elegans*, and *Drosophila*. Preferred genetically modified animals are transgenic animals having a heterologous nucleic acid sequence present as an extrachromosomal element in a portion of its cells, i.e. mosaic animals (see, for example, techniques described by Jakobovits, 1994, Curr. Biol. 4:761-763.) or stably integrated into its germ line DNA (i.e., in the genomic sequence of most or all of its cells). Heterologous nucleic acid is

25   introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal.

Methods of making transgenic animals are well-known in the art (for transgenic mice see Brinster et al., Proc. Nat. Acad. Sci. USA 82: 4438-4442 (1985), U.S. Pat. Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Pat. No. 4,873,191 by Wagner et al.,

30   and Hogan, B., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); for particle bombardment see U.S. Pat. No., 4,945,050, by Sandford *et al.*; for transgenic *Drosophila* see Rubin and Spradling, Science (1982) 218:348-53 and U.S. Pat. No. 4,670,388; for transgenic insects see Berghammer A.J. *et al.*, A Universal Marker for Transgenic Insects (1999) Nature 402:370-371; for transgenic

Zebrafish see Lin S., Transgenic Zebrafish, Methods Mol Biol. (2000);136:375-3830); for

microinjection procedures for fish, amphibian eggs and birds see Houdebine and

Chourrout, Experientia (1991) 47:897-905; for transgenic rats see Hammer *et al.*, Cell

(1990) 63:1099-1112; and for culturing of embryonic stem (ES) cells and the subsequent

5   production of transgenic animals by the introduction of DNA into ES cells using methods

such as electroporation, calcium phosphate/DNA precipitation and direct injection see,

e.g., Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, E. J. Robertson,

ed., IRL Press (1987)). Clones of the nonhuman transgenic animals can be produced

according to available methods (see Wilmut, I. *et al.* (1997) Nature 385:810-813; and PCT

10   International Publication Nos. WO 97/07668 and WO 97/07669).

In one embodiment, the transgenic animal is a "knock-out" animal having a

heterozygous or homozygous alteration in the sequence of an endogenous LCE gene that

results in a decrease of LCE function, preferably such that LCE expression is undetectable

or insignificant. Knock-out animals are typically generated by homologous recombination

15   with a vector comprising a transgene having at least a portion of the gene to be knocked

out. Typically a deletion, addition or substitution has been introduced into the transgene

to functionally disrupt it. The transgene can be a human gene (e.g., from a human

genomic clone) but more preferably is an ortholog of the human gene derived from the

transgenic host species. For example, a mouse LCE gene is used to construct a

20   homologous recombination vector suitable for altering an endogenous LCE gene in the

mouse genome. Detailed methodologies for homologous recombination in mice are

available (see Capecchi, Science (1989) 244:1288-1292; Joyner *et al.*, Nature (1989)

338:153-156). Procedures for the production of non-rodent transgenic mammals and other

animals are also available (Houdebine and Chourrout, *supra*; Pursel *et al.*, Science (1989)

25   244:1281-1288; Simms *et al.*, Bio/Technology (1988) 6:179-183). In a preferred

embodiment, knock-out animals, such as mice harboring a knockout of a specific gene,

may be used to produce antibodies against the human counterpart of the gene that has been

knocked out (Claesson MH et al., (1994) Scan J Immunol 40:257-264; Declerck PJ et

al., (1995) J Biol Chem. 270:8397-400).

30   In another embodiment, the transgenic animal is a "knock-in" animal having an

alteration in its genome that results in altered expression (e.g., increased (including

ectopic) or decreased expression) of the LCE gene, e.g., by introduction of additional

copies of LCE, or by operatively inserting a regulatory sequence that provides for altered

expression of an endogenous copy of the LCE gene. Such regulatory sequences include

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inducible, tissue-specific, and constitutive promoters and enhancer elements. The knock-in can be homozygous or heterozygous.

Transgenic nonhuman animals can also be produced that contain selected systems allowing for regulated expression of the transgene. One example of such a system that

5   may be produced is the cre/loxP recombinase system of bacteriophage P1 (Lakso *et al.*, PNAS (1992) 89:6232-6236; U.S. Pat. No. 4,959,317). If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two

10   transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase. Another example of a recombinase system is the FLP recombinase system of Saccharomyces cerevisiae (O'Gorman et al. (1991) Science 251:1351-1355; U.S. Pat. No. 5,654,182). In a preferred embodiment, both Cre-LoxP and Flp-Frt are used in the same system to regulate expression of the

15   transgene, and for sequential deletion of vector sequences in the same cell (Sun X et al (2000) Nat Genet 25:83-6).

The genetically modified animals can be used in genetic studies to further elucidate the p53 pathway, as animal models of disease and disorders implicating defective p53 function, and for *in vivo* testing of candidate therapeutic agents, such as those identified in

20   screens described below. The candidate therapeutic agents are administered to a genetically modified animal having altered LCE function and phenotypic changes are compared with appropriate control animals such as genetically modified animals that receive placebo treatment, and/or animals with unaltered LCE expression that receive candidate therapeutic agent.

25   In addition to the above-described genetically modified animals having altered LCE function, animal models having defective p53 function (and otherwise normal LCE function), can be used in the methods of the present invention. For example, a p53 knockout mouse can be used to assess, *in vivo*, the activity of a candidate p53 modulating agent identified in one of the *in vitro* assays described below. p53 knockout mice are

30   described in the literature (Jacks et al., Nature 2001;410:1111-1116, 1043-1044; Donehower *et al.*, supra). Preferably, the candidate p53 modulating agent when administered to a model system with cells defective in p53 function, produces a detectable phenotypic change in the model system indicating that the p53 function is restored, i.e., the cells exhibit normal cell cycle progression.

Modulating Agents

The invention provides methods to identify agents that interact with and/or modulate the function of LCE and/or the p53 pathway. Such agents are useful in a variety of diagnostic and therapeutic applications associated with the p53 pathway, as well as in further analysis of the LCE protein and its contribution to the p53 pathway. Accordingly, the invention also provides methods for modulating the p53 pathway comprising the step of specifically modulating LCE activity by administering a LCE-interacting or - modulating agent.

In a preferred embodiment, LCE-modulating agents inhibit or enhance LCE activity or otherwise affect normal LCE function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In a further preferred embodiment, the candidate p53 pathway- modulating agent specifically modulates the function of the LCE. The phrases "specific modulating agent", "specifically modulates", etc., are used herein to refer to modulating agents that directly bind to the LCE polypeptide or nucleic acid, and preferably inhibit, enhance, or otherwise alter, the function of the LCE. The term also encompasses modulating agents that alter the interaction of the LCE with a binding partner or substrate (e.g. by binding to a binding partner of an LCE, or to a protein/binding partner complex, and inhibiting function).

Preferred LCE-modulating agents include small molecule compounds; LCE-interacting proteins, including antibodies and other biotherapeutics; and nucleic acid modulators such as antisense and RNA inhibitors. The modulating agents may be formulated in pharmaceutical compositions, for example, as compositions that may comprise other active ingredients, as in combination therapy, and/or suitable carriers or excipients. Techniques for formulation and administration of the compounds may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, 19th edition.

Small molecule modulators

Small molecules, are often preferred to modulate function of proteins with enzymatic function, and/or containing protein interaction domains. Chemical agents, referred to in the art as "small molecule" compounds are typically organic, non-peptide molecules, having a molecular weight less than 10,000, preferably less than 5,000, more preferably less than 1,000, and most preferably less than 500. This class of modulators includes chemically synthesized molecules, for instance, compounds from combinatorial chemical

libraries. Synthetic compounds may be rationally designed or identified based on known or inferred properties of the LCE protein or may be identified by screening compound libraries. Alternative appropriate modulators of this class are natural products, particularly secondary metabolites from organisms such as plants or fungi, which can also be

5   identified by screening compound libraries for LCE–modulating activity. Methods for generating and obtaining compounds are well known in the art (Schreiber SL, Science (2000) 151: 1964-1969; Radmann J and Gunther J, Science (2000) 151:1947-1948).

Small molecule modulators identified from screening assays, as described below, can be used as lead compounds from which candidate clinical compounds may be designed,

10   optimized, and synthesized. Such clinical compounds may have utility in treating pathologies associated with the p53 pathway. The activity of candidate small molecule modulating agents may be improved several-fold through iterative secondary functional validation, as further described below, structure determination, and candidate modulator modification and testing. Additionally, candidate clinical compounds are generated with

15   specific regard to clinical and pharmacological properties. For example, the reagents may be derivatized and re-screened using *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

Protein Modulators

20   Specific LCE-interacting proteins are useful in a variety of diagnostic and therapeutic applications related to the p53 pathway and related disorders, as well as in validation assays for other LCE-modulating agents. In a preferred embodiment, LCE-interacting proteins affect normal LCE function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In another embodiment, LCE-

25   interacting proteins are useful in detecting and providing information about the function of LCE proteins, as is relevant to p53 related disorders, such as cancer (e.g., for diagnostic means).

An LCE-interacting protein may be endogenous, i.e. one that naturally interacts genetically or biochemically with an LCE, such as a member of the LCE pathway that

30   modulates LCE expression, localization, and/or activity. LCE-modulators include dominant negative forms of LCE-interacting proteins and of LCE proteins themselves. Yeast two-hybrid and variant screens offer preferred methods for identifying endogenous LCE-interacting proteins (Finley, R. L. et al. (1996) in DNA Cloning-Expression Systems: A Practical Approach, eds. Glover D. & Hames B. D (Oxford University Press, Oxford,

England), pp. 169-203; Fashema SF et al., Gene (2000) 250:1-14; Drees BL Curr Opin
Chem Biol (1999) 3:64-70; Vidal M and Legrain P Nucleic Acids Res (1999) 27:919-29;
and U.S. Pat. No. 5,928,868). Mass spectrometry is an alternative preferred method for
the elucidation of protein complexes (reviewed in, e.g., Pandley A and Mann M, Nature
5   (2000) 405:837-846; Yates JR 3rd, Trends Genet (2000) 16:5-8).

An LCE-interacting protein may be an exogenous protein, such as an LCE-specific
antibody or a T-cell antigen receptor (see, e.g., Harlow and Lane (1988) Antibodies, A
Laboratory Manual, Cold Spring Harbor Laboratory; Harlow and Lane (1999) Using
antibodies: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory
10   Press). LCE antibodies are further discussed below.

In preferred embodiments, an LCE-interacting protein specifically binds an LCE
protein. In alternative preferred embodiments, an LCE-modulating agent binds an LCE
substrate, binding partner, or cofactor.

15   *Antibodies*

In another embodiment, the protein modulator is an LCE specific antibody agonist or
antagonist. The antibodies have therapeutic and diagnostic utilities, and can be used in
screening assays to identify LCE modulators. The antibodies can also be used in
dissecting the portions of the LCE pathway responsible for various cellular responses and
20   in the general processing and maturation of the LCE.

Antibodies that specifically bind LCE polypeptides can be generated using known
methods. Preferably the antibody is specific to a mammalian ortholog of LCE
polypeptide, and more preferably, to human LCE. Antibodies may be polyclonal,
monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab
25   fragments, F(ab').sub.2 fragments, fragments produced by a FAb expression library, anti-
idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.
Epitopes of LCE which are particularly antigenic can be selected, for example, by routine
screening of LCE polypeptides for antigenicity or by applying a theoretical method for
selecting antigenic regions of a protein (Hopp and Wood (1981), Proc. Nati. Acad. Sci.
30   U.S.A. 78:3824-28; Hopp and Wood, (1983) Mol. Immunol. 20:483-89; Sutcliffe et al.,
(1983) Science 219:660-66) to the amino acid sequence shown in any of SEQ ID NOs:9,
10, 11, 12, 13, 14, 15, or 16. Monoclonal antibodies with affinities of 10^8 M^{-1} preferably
10^9 M^{-1} to 10^{10} M^{-1}, or stronger can be made by standard procedures as described (Harlow
and Lane, *supra*; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed)

Academic Press, New York; and U.S. Pat. Nos. 4,381,292; 4,451,570; and 4,618,577).
Antibodies may be generated against crude cell extracts of LCE or substantially purified
fragments thereof. If LCE fragments are used, they preferably comprise at least 10, and
more preferably, at least 20 contiguous amino acids of an LCE protein. In a particular

5   embodiment, LCE-specific antigens and/or immunogens are coupled to carrier proteins
that stimulate the immune response. For example, the subject polypeptides are covalently
coupled to the keyhole limpet hemocyanin (KLH) carrier, and the conjugate is emulsified
in Freund's complete adjuvant, which enhances the immune response. An appropriate
immune system such as a laboratory rabbit or mouse is immunized according to

10   conventional protocols.

    The presence of LCE-specific antibodies is assayed by an appropriate assay such as a
solid phase enzyme-linked immunosorbant assay (ELISA) using immobilized
corresponding LCE polypeptides. Other assays, such as radioimmunoassays or
fluorescent assays might also be used.

15   Chimeric antibodies specific to LCE polypeptides can be made that contain different
portions from different animal species. For instance, a human immunoglobulin constant
region may be linked to a variable region of a murine mAb, such that the antibody derives
its biological activity from the human antibody, and its binding specificity from the
murine fragment. Chimeric antibodies are produced by splicing together genes that

20   encode the appropriate regions from each species (Morrison et al., Proc. Natl. Acad. Sci.
(1984) 81:6851-6855; Neuberger et al., Nature (1984) 312:604-608; Takeda et al., Nature
(1985) 31:452-454). Humanized antibodies, which are a form of chimeric antibodies, can
be generated by grafting complementary-determining regions (CDRs) (Carlos, T. M., J. M.
Harlan. 1994. Blood 84:2068-2101) of mouse antibodies into a background of human

25   framework regions and constant regions by recombinant DNA technology (Riechmann
LM, et al., 1988 Nature 323: 323-327). Humanized antibodies contain ~10% murine
sequences and ~90% human sequences, and thus further reduce or eliminate
immunogenicity, while retaining the antibody specificities (Co MS, and Queen C. 1991
Nature 351: 501-501; Morrison SL. 1992 Ann. Rev. Immun. 10:239-265). Humanized

30   antibodies and methods of their production are well-known in the art (U.S. Pat. Nos.
5,530,101, 5,585,089, 5,693,762, and 6,180,370).

    LCE-specific single chain antibodies which are recombinant, single chain polypeptides
formed by linking the heavy and light chain fragments of the Fv regions via an amino acid
bridge, can be produced by methods known in the art (U.S. Pat. No. 4,946,778; Bird,

Science (1988) 242:423-426; Huston et al., Proc. Natl. Acad. Sci. USA (1988) 85:5879-5883; and Ward et al., Nature (1989) 334:544-546).

Other suitable techniques for antibody production involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of

5   antibodies in phage or similar vectors (Huse et al., Science (1989) 246:1275-1281). As used herein, T-cell antigen receptors are included within the scope of antibody modulators (Harlow and Lane, 1988, *supra*).

The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, antibodies will be labeled by joining, either covalently or non-

10   covalently, a substance that provides for a detectable signal, or that is toxic to cells that express the targeted protein (Menard S, et al., Int J. Biol Markers (1989) 4:131-134). A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, fluorescent emitting lanthanide

15   metals, chemiluminescent moieties, bioluminescent moieties, magnetic particles, and the like (U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241). Also, recombinant immunoglobulins may be produced (U.S. Pat. No. 4,816,567). Antibodies to cytoplasmic polypeptides may be delivered and reach their targets by conjugation with membrane-penetrating toxin proteins (U.S. Pat. No.

20   6,086,900).

When used therapeutically in a patient, the antibodies of the subject invention are typically administered parenterally, when possible at the target site, or intravenously. The therapeutically effective dose and dosage regimen is determined by clinical studies. Typically, the amount of antibody administered is in the range of about 0.1 mg/kg –to

25   about 10 mg/kg of patient weight. For parenteral administration, the antibodies are formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion) in association with a pharmaceutically acceptable vehicle. Such vehicles are inherently nontoxic and non-therapeutic. Examples are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils, ethyl

30   oleate, or liposome carriers may also be used. The vehicle may contain minor amounts of additives, such as buffers and preservatives, which enhance isotonicity and chemical stability or otherwise enhance therapeutic potential. The antibodies' concentrations in such vehicles are typically in the range of about 1 mg/ml to about10 mg/ml.

Immunotherapeutic methods are further described in the literature (US Pat. No. 5,859,206; WO0073469).

Specific biotherapeutics

5   In a preferred embodiment, an LCE-interacting protein may have biotherapeutic applications. Biotherapeutic agents formulated in pharmaceutically acceptable carriers and dosages may be used to activate or inhibit signal transduction pathways. This modulation may be accomplished by binding a ligand, thus inhibiting the activity of the pathway; or by binding a receptor, either to inhibit activation of, or to activate, the

10   receptor. Alternatively, the biotherapeutic may itself be a ligand capable of activating or inhibiting a receptor. Biotherapeutic agents and methods of producing them are described in detail in U.S. Pat. No. 6,146,628.

LCE ligand(s), antibodies to the ligand(s) or the LCE itself may be used as biotherapeutics to modulate the activity of LCE in the p53 pathway.

15

Nucleic Acid Modulators

Other preferred LCE-modulating agents comprise nucleic acid molecules, such as antisense oligomers or double stranded RNA (dsRNA), which generally inhibit LCE activity. Preferred nucleic acid modulators interfere with the function of the LCE nucleic

20   acid such as DNA replication, transcription, translocation of the LCE RNA to the site of protein translation, translation of protein from the LCE RNA, splicing of the LCE RNA to yield one or more mRNA species, or catalytic activity which may be engaged in or facilitated by the LCE RNA.

In one embodiment, the antisense oligomer is an oligonucleotide that is sufficiently

25   complementary to an LCE mRNA to bind to and prevent translation, preferably by binding to the 5′ untranslated region. LCE-specific antisense oligonucleotides, preferably range from at least 6 to about 200 nucleotides. In some embodiments the oligonucleotide is preferably at least 10, 15, or 20 nucleotides in length. In other embodiments, the oligonucleotide is preferably less than 50, 40, or 30 nucleotides in length. The

30   oligonucleotide can be DNA or RNA or a chimeric mixture or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include other appending groups such as peptides, agents that facilitate transport across the cell membrane, hybridization-triggered cleavage agents, and intercalating agents.

In another embodiment, the antisense oligomer is a phosphothioate morpholino . oligomer (PMO). PMOs are assembled from four different morpholino subunits, each of which contain one of four genetic bases (A, C, G, or T) linked to a six-membered morpholine ring. Polymers of these subunits are joined by non-ionic phosphodiamidate

5   intersubunit linkages. Details of how to make and use PMOs and other antisense oligomers are well known in the art (e.g. see WO99/18193; Probst JC, Antisense Oligodeoxynucleotide and Ribozyme Design, Methods. (2000) 22(3):271-281; Summerton J, and Weller D. 1997 Antisense Nucleic Acid Drug Dev. :7:187-95; US Pat. No. 5,235,033; and US Pat No. 5,378,841).

10   Alternative preferred LCE nucleic acid modulators are double-stranded RNA species mediating RNA interference (RNAi). RNAi is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. Methods relating to the use of RNAi to silence genes in *C. elegans, Drosophila*, plants, and humans are known in the

15   art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); Sharp, P. A. RNA interference 2001. Genes Dev. 15, 485-490 (2001); Hammond, S. M., et al., Nature Rev. Genet. 2, 110-1119 (2001); Tuschl, T. Chem. Biochem. 2, 239-245 (2001); Hamilton, A. et al., Science 286, 950-952 (1999); Hammond, S. M., et al., Nature 404, 293-296 (2000); Zamore, P. D., et al., Cell 101, 25-33 (2000); Bernstein, E.,

20   et al., Nature 409, 363-366 (2001); Elbashir, S. M., et al., Genes Dev. 15, 188-200 (2001); WO0129058; WO9932619; Elbashir SM, et al., 2001 Nature 411:494-498).

Nucleic acid modulators are commonly used as research reagents, diagnostics, and therapeutics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used to elucidate the function of particular

25   genes (see, for example, U.S. Pat. No. 6,165,790). Nucleic acid modulators are also used, for example, to distinguish between functions of various members of a biological pathway. For example, antisense oligomers have been employed as therapeutic moieties in the treatment of disease states in animals and man and have been demonstrated in numerous clinical trials to be safe and effective (Milligan JF, *et al*, Current Concepts in Antisense

30   Drug Design, J Med Chem. (1993) 36:1923-1937; Tonkinson JL *et al*., Antisense Oligodeoxynucleotides as Clinical Therapeutic Agents, Cancer Invest. (1996) 14:54-65). Accordingly, in one aspect of the invention, an LCE-specific nucleic acid modulator is used in an assay to further elucidate the role of the LCE in the p53 pathway, and/or its relationship to other members of the pathway. In another aspect of the invention, an LCE-

specific antisense oligomer is used as a therapeutic agent for treatment of p53-related disease states.

Assay Systems

5   The invention provides assay systems and screening methods for identifying specific modulators of LCE activity. As used herein, an "assay system" encompasses all the components required for performing and analyzing results of an assay that detects and/or measures a particular event. In general, primary assays are used to identify or confirm a modulator's specific biochemical or molecular effect with respect to the LCE nucleic acid

10   or protein. In general, secondary assays further assess the activity of a LCE modulating agent identified by a primary assay and may confirm that the modulating agent affects LCE in a manner relevant to the p53 pathway. In some cases, LCE modulators will be directly tested in a secondary assay.

In a preferred embodiment, the screening method comprises contacting a suitable

15   assay system comprising an LCE polypeptide with a candidate agent under conditions whereby, but for the presence of the agent, the system provides a reference activity (e.g. binding activity), which is based on the particular molecular event the screening method detects. A statistically significant difference between the agent-biased activity and the reference activity indicates that the candidate agent modulates LCE activity, and hence the

20   p53 pathway.

Primary Assays

The type of modulator tested generally determines the type of primary assay.

25   *Primary assays for small molecule modulators*

For small molecule modulators, screening assays are used to identify candidate modulators. Screening assays may be cell-based or may use a cell-free system that recreates or retains the relevant biochemical reaction of the target protein (reviewed in Sittampalam GS *et al.*, Curr Opin Chem Biol (1997) 1:384-91 and accompanying

30   references). As used herein the term "cell-based" refers to assays using live cells, dead cells, or a particular cellular fraction, such as a membrane, endoplasmic reticulum, or mitochondrial fraction. The term "cell free" encompasses assays using substantially purified protein (either endogenous or recombinantly produced), partially purified or crude cellular extracts. Screening assays may detect a variety of molecular events, including

protein-DNA interactions, protein-protein interactions (*e.g.*, receptor-ligand binding), transcriptional activity (*e.g.*, using a reporter gene), enzymatic activity (*e.g.*, via a property of the substrate), activity of second messengers, immunogenicty and changes in cellular morphology or other cellular characteristics. Appropriate screening assays may use a wide

5   range of detection methods including fluorescent, radioactive, colorimetric, spectrophotometric, and amperometric methods, to provide a read-out for the particular molecular event detected.

Cell-based screening assays usually require systems for recombinant expression of LCE and any auxiliary proteins demanded by the particular assay. Appropriate methods

10   for generating recombinant proteins produce sufficient quantities of proteins that retain their relevant biological activities and are of sufficient purity to optimize activity and assure assay reproducibility. Yeast two-hybrid and variant screens, and mass spectrometry provide preferred methods for determining protein-protein interactions and elucidation of protein complexes. In certain applications, when LCE-interacting proteins are used in

15   screens to identify small molecule modulators, the binding specificity of the interacting protein to the LCE protein may be assayed by various known methods such as substrate processing (e.g. ability of the candidate LCE-specific binding agents to function as negative effectors in LCE-expressing cells), binding equilibrium constants (usually at least about 10^7 M^{-1}, preferably at least about 10^8 M^{-1}, more preferably at least about 10^9 M^{-1}),

20   and immunogenicity (e.g. ability to elicit LCE specific antibody in a heterologous host such as a mouse, rat, goat or rabbit). For enzymes and receptors, binding may be assayed by, respectively, substrate and ligand processing.

The screening assay may measure a candidate agent's ability to specifically bind to or modulate activity of a LCE polypeptide, a fusion protein thereof, or to cells or membranes

25   bearing the polypeptide or fusion protein. The LCE polypeptide can be full length or a fragment thereof that retains functional LCE activity. The LCE polypeptide may be fused to another polypeptide, such as a peptide tag for detection or anchoring, or to another tag. The LCE polypeptide is preferably human LCE, or is an ortholog or derivative thereof as described above. In a preferred embodiment, the screening assay detects candidate agent-

30   based modulation of LCE interaction with a binding target, such as an endogenous or exogenous protein or other substrate that has LCE –specific binding activity, and can be used to assess normal LCE gene function.

Suitable assay formats that may be adapted to screen for LCE modulators are known in the art. Preferred screening assays are high throughput or ultra high throughput and thus

provide automated, cost-effective means of screening compound libraries for lead
compounds (Fernandes PB, Curr Opin Chem Biol (1998) 2:597-603; Sundberg SA, Curr
Opin Biotechnol 2000, 11:47-53). In one preferred embodiment, screening assays uses
fluorescence technologies, including fluorescence polarization, time-resolved

5   fluorescence, and fluorescence resonance energy transfer. These systems offer means to
monitor protein-protein or DNA-protein interactions in which the intensity of the signal
emitted from dye-labeled molecules depends upon their interactions with partner
molecules (*e.g.,* Selvin PR, Nat Struct Biol (2000) 7:730-4; Fernandes PB, *supra*;
Hertzberg RP and Pope AJ, Curr Opin Chem Biol (2000) 4:445-451).

10   A variety of suitable assay systems may be used to identify candidate LCE and p53
pathway modulators (e.g. U.S. Pat. No. 6,020,135 (p53 modulation)).   Specific preferred
assays are described in more detail below.

Elongase assays. Assays for elongases are well-known in the art. In one example, an
15   elongation assay uses chromatographic (e.g., HPLC) analysis of labeled elongation
products to assess LCE activity. Long chain fatty acids may be labeled with 14-C (Moon
YA et al. (2001) J Biol Chem 276:45358-45366). Briefly, fatty acid elongation activity is
measured in microsomes prepared from transfected cells. Fatty acyl-CoAs or BSA bound
fatty acids may be used as substrates for the reactions. Substrate mixtures also include [2-
20   ^{14}C]malonyl-CoA. Elongation reactions are initiated when microsomal proteins and
substrates are mixed. After termination of the reactions, fatty acids are collected, washed,
and counted. Elongase activity is measured and expressed as the amount of radioactivity
incorporated into the fatty acids.

25   **Apoptosis assays.** Assays for apoptosis may be performed by terminal
deoxynucleotidyl transferase-mediated digoxigenin-11-dUTP nick end labeling (TUNEL)
assay. The TUNEL assay is used to measure nuclear DNA fragmentation characteristic of
apoptosis (Lazebnik *et al.*, 1994, Nature 371, 346), by following the incorporation of
fluorescein-dUTP (Yonehara *et al.*, 1989, J. Exp. Med. 169, 1747). Apoptosis may further
30   be assayed by acridine orange staining of tissue culture cells (Lucas, R., et al., 1998, Blood
15:4730-41). An apoptosis assay system may comprise a cell that expresses an LCE, and
that optionally has defective p53 function (e.g. p53 is over-expressed or under-expressed
relative to wild-type cells). A test agent can be added to the apoptosis assay system and
changes in induction of apoptosis relative to controls where no test agent is added, identify

candidate p53 modulating agents. In some embodiments of the invention, an apoptosis
assay may be used as a secondary assay to test a candidate p53 modulating agents that is
initially identified using a cell-free assay system. An apoptosis assay may also be used to
test whether LCE function plays a direct role in apoptosis. For example, an apoptosis

5   assay may be performed on cells that over- or under-express LCE relative to wild type
cells. Differences in apoptotic response compared to wild type cells suggests that the LCE
plays a direct role in the apoptotic response. Apoptosis assays are described further in US
Pat. No. 6,133,437.

10   **Cell proliferation and cell cycle assays.** Cell proliferation may be assayed via
bromodeoxyuridine (BRDU) incorporation. This assay identifies a cell population
undergoing DNA synthesis by incorporation of BRDU into newly-synthesized DNA.
Newly-synthesized DNA may then be detected using an anti-BRDU antibody (Hoshino *et
al.*, 1986, Int. J. Cancer 38, 369; Campana *et al.*, 1988, J. Immunol. Meth. 107, 79), or by

15   other means.
Cell Proliferation may also be examined using [^{3}H]-thymidine incorporation (Chen, J.,
1996, Oncogene 13:1395-403; Jeoung, J., 1995, J. Biol. Chem. 270:18367-73). This
assay allows for quantitative characterization of S-phase DNA syntheses. In this assay,
cells synthesizing DNA will incorporate [^{3}H]-thymidine into newly synthesized DNA.

20   Incorporation can then be measured by standard techniques such as by counting of
radioisotope in a scintillation counter (e.g., Beckman LS 3800 Liquid Scintillation
Counter).
Cell proliferation may also be assayed by colony formation in soft agar (Sambrook et
al., Molecular Cloning, Cold Spring Harbor (1989)). For example, cells transformed with

25   LCE are seeded in soft agar plates, and colonies are measured and counted after two
weeks incubation.
Involvement of a gene in the cell cycle may be assayed by flow cytometry (Gray JW et
al. (1986) Int J Radiat Biol Relat Stud Phys Chem Med 49:237-55). Cells transfected with
an LCE may be stained with propidium iodide and evaluated in a flow cytometer

30   (available from Becton Dickinson).
Accordingly, a cell proliferation or cell cycle assay system may comprise a cell that
expresses an LCE, and that optionally has defective p53 function (e.g. p53 is over-
expressed or under-expressed relative to wild-type cells). A test agent can be added to the
assay system and changes in cell proliferation or cell cycle relative to controls where no

test agent is added, identify candidate p53 modulating agents. In some embodiments of the invention, the cell proliferation or cell cycle assay may be used as a secondary assay to test a candidate p53 modulating agents that is initially identified using another assay system such as a cell-free assay system. A cell proliferation assay may also be used to test

5   whether LCE function plays a direct role in cell proliferation or cell cycle. For example, a cell proliferation or cell cycle assay may be performed on cells that over- or under-express LCE relative to wild type cells. Differences in proliferation or cell cycle compared to wild type cells suggests that the LCE plays a direct role in cell proliferation or cell cycle.

10   **Angiogenesis.** Angiogenesis may be assayed using various human endothelial cell systems, such as umbilical vein, coronary artery, or dermal cells. Suitable assays include Alamar Blue based assays (available from Biosource International) to measure proliferation; migration assays using fluorescent molecules, such as the use of Becton Dickinson Falcon HTS FluoroBlock cell culture inserts to measure migration of cells

15   through membranes in presence or absence of angiogenesis enhancer or suppressors; and tubule formation assays based on the formation of tubular structures by endothelial cells on Matrigel® (Becton Dickinson). Accordingly, an angiogenesis assay system may comprise a cell that expresses an LCE, and that optionally has defective p53 function (e.g. p53 is over-expressed or under-expressed relative to wild-type cells). A test agent can be

20   added to the angiogenesis assay system and changes in angiogenesis relative to controls where no test agent is added, identify candidate p53 modulating agents. In some embodiments of the invention, the angiogenesis assay may be used as a secondary assay to test a candidate p53 modulating agents that is initially identified using another assay system. An angiogenesis assay may also be used to test whether LCE function plays a

25   direct role in cell proliferation. For example, an angiogenesis assay may be performed on cells that over- or under-express LCE relative to wild type cells. Differences in angiogenesis compared to wild type cells suggests that the LCE plays a direct role in angiogenesis.

30   **Hypoxic induction.** The alpha subunit of the transcription factor, hypoxia inducible factor-1 (HIF-1), is upregulated in tumor cells following exposure to hypoxia in vitro. Under hypoxic conditions, HIF-1 stimulates the expression of genes known to be important in tumour cell survival, such as those encoding glyolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells

26

transfected with LCE in hypoxic conditions (such as with 0.1% O2, 5% CO2, and balance N2, generated in a Napco 7001 incubator (Precision Scientific)) and normoxic conditions, followed by assessment of gene activity or expression by Taqman®. For example, a hypoxic induction assay system may comprise a cell that expresses an LCE, and that

5   optionally has a mutated p53 (e.g. p53 is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the hypoxic induction assay system and changes in hypoxic response relative to controls where no test agent is added, identify candidate p53 modulating agents. In some embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate p53 modulating

10   agents that is initially identified using another assay system. A hypoxic induction assay may also be used to test whether LCE function plays a direct role in the hypoxic response. For example, a hypoxic induction assay may be performed on cells that over- or under-express LCE relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the LCE plays a direct role in hypoxic induction.

15

Cell adhesion. Cell adhesion assays measure adhesion of cells to purified adhesion proteins, or adhesion of cells to each other, in presence or absence of candidate modulating agents. Cell-protein adhesion assays measure the ability of agents to modulate the adhesion of cells to purified proteins. For example, recombinant proteins are

20   produced, diluted to 2.5g/mL in PBS, and used to coat the wells of a microtiter plate. The wells used for negative control are not coated. Coated wells are then washed, blocked with 1% BSA, and washed again. Compounds are diluted to 2x final test concentration and added to the blocked, coated wells. Cells are then added to the wells, and the unbound cells are washed off. Retained cells are labeled directly on the plate by adding a

25   membrane-permeable fluorescent dye, such as calcein-AM, and the signal is quantified in a fluorescent microplate reader.

Cell-cell adhesion assays measure the ability of agents to modulate binding of cell adhesion proteins with their native ligands. These assays use cells that naturally or recombinantly express the adhesion protein of choice. In an exemplary assay, cells

30   expressing the cell adhesion protein are plated in wells of a multiwell plate. Cells expressing the ligand are labeled with a membrane-permeable fluorescent dye, such as BCECF , and allowed to adhere to the monolayers in the presence of candidate agents. Unbound cells are washed off, and bound cells are detected using a fluorescence plate reader.

27

High-throughput cell adhesion assays have also been described. In one such assay,
small molecule ligands and peptides are bound to the surface of microscope slides using a
microarray spotter, intact cells are then contacted with the slides, and unbound cells are
washed off. In this assay, not only the binding specificity of the peptides and modulators

5   against cell lines are determined, but also the functional cell signaling of attached cells
using immunofluorescence techniques in situ on the microchip is measured (Falsey JR et
al., Bioconjug Chem. 2001 May-Jun;12(3):346-53).

Tubulogenesis. Tubulogenesis assays monitor the ability of cultured cells, generally

10   endothelial cells, to form tubular structures on a matrix substrate, which generally
simulates the environment of the extracellular matrix. Exemplary substrates include
Matrigel™ (Becton Dickinson), an extract of basement membrane proteins containing
laminin, collagen IV, and heparin sulfate proteoglycan, which is liquid at 4°C and forms a
solid gel at 37°C. Other suitable matrices comprise extracellular components such as

15   collagen, fibronectin, and/or fibrin. Cells are stimulated with a pro-angiogenic stimulant,
and their ability to form tubules is detected by imaging. Tubules can generally be detected
after an overnight incubation with stimuli, but longer or shorter time frames may also be
used. Tube formation assays are well known in the art (e.g., Jones MK et al., 1999, Nature
Medicine 5:1418-1423). These assays have traditionally involved stimulation with serum

20   or with the growth factors FGF or VEGF. Serum represents an undefined source of
growth factors. In a preferred embodiment, the assay is performed with cells cultured in
serum free medium, in order to control which process or pathway a candidate agent
modulates. Moreover, we have found that different target genes respond differently to
stimulation with different pro-angiogenic agents, including inflammatory angiogenic

25   factors such as TNF-alpa. Thus, in a further preferred embodiment, a tubulogenesis assay
system comprises testing an LCE's response to a variety of factors, such as FGF, VEGF,
phorbol myristate acetate (PMA), TNF-alpha, ephrin, etc.

Cell Migration. An invasion/migration assay (also called a migration assay) tests the

30   ability of cells to overcome a physical barrier and to migrate towards pro-angiogenic
signals. Migration assays are known in the art (e.g., Paik JH et al., 2001, J Biol Chem
276:11830-11837). In a typical experimental set-up, cultured endothelial cells are seeded
onto a matrix-coated porous lamina, with pore sizes generally smaller than typical cell
size. The matrix generally simulates the environment of the extracellular matrix, as

described above. The lamina is typically a membrane, such as the transwell polycarbonate membrane (Corning Costar Corporation, Cambridge, MA), and is generally part of an upper chamber that is in fluid contact with a lower chamber containing pro-angiogenic stimuli. Migration is generally assayed after an overnight incubation with stimuli, but

5   longer or shorter time frames may also be used. Migration is assessed as the number of cells that crossed the lamina, and may be detected by staining cells with hemotoxylin solution (VWR Scientific, South San Francisco, CA), or by any other method for determining cell number. In another exemplary set up, cells are fluorescently labeled and migration is detected using fluorescent readings, for instance using the Falcon HTS

10   FluoroBlok (Becton Dickinson). While some migration is observed in the absence of stimulus, migration is greatly increased in response to pro-angiogenic factors. As described above, a preferred assay system for migration/invasion assays comprises testing an LCE's response to a variety of pro-angiogenic factors, including tumor angiogenic and inflammatory angiogenic agents, and culturing the cells in serum free medium.

15

Sprouting assay. A sprouting assay is a three-dimensional *in vitro* angiogenesis assay that uses a cell-number defined spheroid aggregation of endothelial cells ("spheroid"), embedded in a collagen gel-based matrix. The spheroid can serve as a starting point for the sprouting of capillary-like structures by invasion into the

20   extracellular matrix (termed "cell sprouting") and the subsequent formation of complex anastomosing networks (Korff and Augustin, 1999, J Cell Sci 112:3249-58). In an exemplary experimental set-up, spheroids are prepared by pipetting 400 human umbilical vein endothelial cells into individual wells of a nonadhesive 96-well plates to allow overnight spheroidal aggregation (Korff and Augustin: J Cell Biol 143: 1341-52, 1998).

25   Spheroids are harvested and seeded in 900μl of methocel-collagen solution and pipetted into individual wells of a 24 well plate to allow collagen gel polymerization. Test agents are added after 30 min by pipetting 100 μl of 10-fold concentrated working dilution of the test substances on top of the gel. Plates are incubated at 37°C for 24h. Dishes are fixed at the end of the experimental incubation period by addition of paraformaldehyde. Sprouting

30   intensity of endothelial cells can be quantitated by an automated image analysis system to determine the cumulative sprout length per spheroid.

Primary assays for antibody modulators

For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the LCE protein. Methods for testing antibody affinity and specificity are well known in the art (Harlow and Lane, 1988, 1999, *supra*).

5   The enzyme-linked immunosorbant assay (ELISA) is a preferred method for detecting LCE-specific antibodies; others include FACS assays, radioimmunoassays, and fluorescent assays.

Primary assays for nucleic acid modulators

10 For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance LCE gene expression, preferably mRNA expression. In general, expression analysis comprises comparing LCE expression in like populations of cells (*e.g.*, two pools of cells that endogenously or recombinantly express LCE) in the presence and absence of the nucleic acid modulator. Methods for analyzing mRNA and

15 protein expression are well known in the art. For instance, Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR (*e.g.*, using the TaqMan®, PE Applied Biosystems), or microarray analysis may be used to confirm that LCE mRNA expression is reduced in cells treated with the nucleic acid modulator (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, *eds.*, John Wiley & Sons, Inc., chapter 4;

20 Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm DH and Guiseppi-Elie, A Curr Opin Biotechnol 2001, 12:41-47). Protein expression may also be monitored. Proteins are most commonly detected with specific antibodies or antisera directed against either the LCE protein or specific peptides. A variety of means including Western blotting, ELISA, or in situ detection, are available

25 (Harlow E and Lane D, 1988 and 1999, *supra*).

Secondary Assays

Secondary assays may be used to further assess the activity of LCE-modulating agent identified by any of the above methods to confirm that the modulating agent affects LCE

30 in a manner relevant to the p53 pathway. As used herein, LCE-modulating agents encompass candidate clinical compounds or other agents derived from previously identified modulating agent. Secondary assays can also be used to test the activity of a modulating agent on a particular genetic or biochemical pathway or to test the specificity of the modulating agent's interaction with LCE.

Secondary assays generally compare like populations of cells or animals (*e.g.*, two pools of cells or animals that endogenously or recombinantly express LCE) in the presence and absence of the candidate modulator. In general, such assays test whether treatment of cells or animals with a candidate LCE–modulating agent results in changes in the p53

5   pathway in comparison to untreated (or mock- or placebo-treated) cells or animals. Certain assays use "sensitized genetic backgrounds", which, as used herein, describe cells or animals engineered for altered expression of genes in the p53 or interacting pathways.

Cell-based assays

10   Cell based assays may use a variety of mammalian cell lines known to have defective p53 function (e.g. SAOS-2 osteoblasts, H1299 lung cancer cells, C33A and HT3 cervical cancer cells, HT-29 and DLD-1 colon cancer cells, among others, available from American Type Culture Collection (ATCC), Manassas, VA). Cell based assays may detect endogenous p53 pathway activity or may rely on recombinant expression of p53

15   pathway components. Any of the aforementioned assays may be used in this cell-based format. Candidate modulators are typically added to the cell media but may also be injected into cells or delivered by any other efficacious means.

Animal Assays

20   A variety of non-human animal models of normal or defective p53 pathway may be used to test candidate LCE modulators. Models for defective p53 pathway typically use genetically modified animals that have been engineered to mis-express (*e.g.*, over-express or lack expression in) genes involved in the p53 pathway. Assays generally require systemic delivery of the candidate modulators, such as by oral administration, injection,

25   etc.

In a preferred embodiment, p53 pathway activity is assessed by monitoring neovascularization and angiogenesis. Animal models with defective and normal p53 are used to test the candidate modulator's affect on LCE in Matrigel® assays. Matrigel® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen

30   IV, and heparin sulfate proteoglycan. It is provided as a sterile liquid at 4° C, but rapidly forms a solid gel at 37° C. Liquid Matrigel® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the LCE. The mixture is then injected subcutaneously(SC) into female athymic nude mice (Taconic, Germantown, NY) to support an intense vascular response. Mice with Matrigel® pellets

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may be dosed via oral (PO), intraperitoneal (IP), or intravenous (IV) routes with the candidate modulator. Mice are euthanized 5 - 12 days post-injection, and the Matrigel® pellet is harvested for hemoglobin analysis (Sigma plasma hemoglobin kit). Hemoglobin content of the gel is found to correlate the degree of neovascularization in the gel.

5   In another preferred embodiment, the effect of the candidate modulator on LCE is assessed via tumorigenicity assays. In one example, xenograft human tumors are implanted SC into female athymic mice, 6-7 week old, as single cell suspensions either from a pre-existing tumor or from *in vitro* culture. The tumors which express the LCE endogenously are injected in the flank, 1×10^5 to 1×10^7 cells per mouse in a volume of

10   100 μL using a 27gauge needle. Mice are then ear tagged and tumors are measured twice weekly. Candidate modulator treatment is initiated on the day the mean tumor weight reaches 100 mg. Candidate modulator is delivered IV, SC, IP, or PO by bolus administration. Depending upon the pharmacokinetics of each unique candidate modulator, dosing can be performed multiple times per day. The tumor weight is assessed

15   by measuring perpendicular diameters with a caliper and calculated by multiplying the measurements of diameters in two dimensions. At the end of the experiment, the excised tumors maybe utilized for biomarker identification or further analyses. For immunohistochemistry staining, xenograft tumors are fixed in 4% paraformaldehyde, 0.1M phosphate, pH 7.2, for 6 hours at 4°C, immersed in 30% sucrose in PBS, and rapidly

20   frozen in isopentane cooled with liquid nitrogen.

Diagnostic and therapeutic uses

Specific LCE-modulating agents are useful in a variety of diagnostic and therapeutic applications where disease or disease prognosis is related to defects in the p53 pathway,

25   such as angiogenic, apoptotic, or cell proliferation disorders. Accordingly, the invention also provides methods for modulating the p53 pathway in a cell, preferably a cell pre-determined to have defective p53 function, comprising the step of administering an agent to the cell that specifically modulates LCE activity. Preferably, the modulating agent produces a detectable phenotypic change in the cell indicating that the p53 function is

30   restored, i.e., for example, the cell undergoes normal proliferation or progression through the cell cycle.

The discovery that LCE is implicated in p53 pathway provides for a variety of methods that can be employed for the diagnostic and prognostic evaluation of diseases and

disorders involving defects in the p53 pathway and for the identification of subjects having a predisposition to such diseases and disorders.

Various expression analysis methods can be used to diagnose whether LCE expression occurs in a particular sample, including Northern blotting, slot blotting, ribonuclease

5 protection, quantitative RT-PCR, and microarray analysis. (e.g., Current Protocols in Molecular Biology (1994) Ausubel FM et al., eds., John Wiley & Sons, Inc., chapter 4; Freeman WM et al., Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm and Guiseppi-Elie, Curr Opin Biotechnol 2001, 12:41-47). Tissues having a disease or disorder implicating defective p53 signaling that express an LCE, are

10 identified as amenable to treatment with an LCE modulating agent. In a preferred application, the p53 defective tissue overexpresses an LCE relative to normal tissue. For example, a Northern blot analysis of mRNA from tumor and normal cell lines, or from tumor and matching normal tissue samples from the same patient, using full or partial LCE cDNA sequences as probes, can determine whether particular tumors express or

15 overexpress LCE. Alternatively, the TaqMan® is used for quantitative RT-PCR analysis of LCE expression in cell lines, normal tissues and tumor samples (PE Applied Biosystems).

Various other diagnostic methods may be performed, for example, utilizing reagents such as the LCE oligonucleotides, and antibodies directed against an LCE, as described

20 above for: (1) the detection of the presence of LCE gene mutations, or the detection of either over- or under-expression of LCE mRNA relative to the non-disorder state; (2) the detection of either an over- or an under-abundance of LCE gene product relative to the non-disorder state; and (3) the detection of perturbations or abnormalities in the signal transduction pathway mediated by LCE.

25 Thus, in a specific embodiment, the invention is drawn to a method for diagnosing a disease in a patient, the method comprising: a) obtaining a biological sample from the patient; b) contacting the sample with a probe for LCE expression; c) comparing results from step (b) with a control; and d) determining whether step (c) indicates a likelihood of disease. Preferably, the disease is cancer, most preferably a cancer as shown in TABLE 1.

30 The probe may be either DNA or protein, including an antibody.

EXAMPLES

The following experimental section and examples are offered by way of illustration and not by way of limitation.

33

I. Drosophila p53 screen

The Drosophila p53 gene was overexpressed specifically in the wing using the vestigial margin quadrant enhancer. Increasing quantities of Drosophila p53 (titrated using different strength transgenic inserts in 1 or 2 copies) caused deterioration of normal

5   wing morphology from mild to strong, with phenotypes including disruption of pattern and polarity of wing hairs, shortening and thickening of wing veins, progressive crumpling of the wing and appearance of dark "death" inclusions in wing blade. In a screen designed to identify enhancers and suppressors of Drosophila p53, homozygous females carrying two copies of p53 were crossed to 5663 males carrying random insertions of a piggyBac

10   transposon (Fraser M et al., Virology (1985) 145:356-361). Progeny containing insertions were compared to non-insertion-bearing sibling progeny for enhancement or suppression of the p53 phenotypes. Sequence information surrounding the piggyBac insertion site was used to identify the modifier genes. Modifiers of the wing phenotype were identified as members of the p53 pathway. baldspot was an enhancer of the wing phenotype. Human

15   orthologs of the modifiers are referred to herein as LCE.

BLAST analysis (Altschul et al., supra) was employed to identify Targets from Drosophila modifiers. [For example, representative sequences from LCE, GI#s 10444345, 13129088, 12232379, and 17454617 (SEQ ID NOs:9, 11, 14, and 16, respectivley) share 45%, 48%, 25%, and 41% amino acid identity, respectively, with the Drosophila.baldspot.

20   Various domains, signals, and functional subunits in proteins were analyzed using the PSORT (Nakai K., and Horton P., Trends Biochem Sci, 1999, 24:34-6; Kenta Nakai, Protein sorting signals and prediction of subcellular localization, Adv. Protein Chem. 54, 277-344 (2000)), PFAM (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2; http://pfam.wustl.edu), SMART (Ponting CP, et al., SMART: identification and annotation

25   of domains from signaling and extracellular protein sequences. Nucleic Acids Res. 1999 Jan 1;27(1):229-32), TM-HMM (Erik L.L. Sonnhammer, Gunnar von Heijne, and Anders Krogh: A hidden Markov model for predicting transmembrane helices in protein sequences. In Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, p 175-182 Ed J. Glasgow, T. Littlejohn, F. Major, R. Lathrop, D. Sankoff, and C. Sensen

30   Menlo Park, CA: AAAI Press, 1998), and clust (Remm M, and Sonnhammer E. Classification of transmembrane protein families in the Caenorhabditis elegans genome and identification of human orthologs. Genome Res. 2000 Nov;10(11):1679-89) programs. Using PFAM, GNS1/SUR4 domain (PFAM 01151) of LCE from GI#s 10444345, 13129088, 12232379, and 17454617 (SEQ ID NOs:9, 11, 14, and 16, respectively) are

located respectively at approximately amino acid residues 1-235, 10 to 265, 9 to 289, and

55 to 270. Further, using TM-HMM, GI# 10444345 (SEQ ID NO:9) has 7 transmembrane

domains with start and end amino acids of (4,21) (26,48) (52,74) (81,103) (131,153)

(166,188) (203,225); GI# 13129088 (SEQ ID NO:11) has 6 transmembrane domains with

5   start and end coordinates of (34,51) (66,88) (137,156) (161,183) (195,217) (232,254);

GI#12232379 (SEQ ID NO:14) has 7 transmembrane domains with start and end

coordinates of (47,64) (77,99) (125,147) (154,173) (183,205) (217,239) (249,267); and

GI#17454617 (SEQ ID NO:16) has 7 transmembrane domains with start and end

coordinates of (33,55) (60,82) (86,108) (115,137) (165,187) (200,222) (237,259).

10

II.   High-Throughput In Vitro Fluorescence Polarization Assay

Fluorescently-labeled LCE peptide/substrate are added to each well of a 96-well

microtiter plate, along with a test agent in a test buffer (10 mM HEPES, 10 mM NaCl, 6

mM magnesium chloride, pH 7.6). Changes in fluorescence polarization, determined by

15   using a Fluorolite FPM-2 Fluorescence Polarization Microtiter System (Dynatech

Laboratories, Inc), relative to control values indicates the test compound is a candidate

modifier of LCE activity.

III.   High-Throughput In Vitro Binding Assay.

20   ^{33}P-labeled LCE peptide is added in an assay buffer (100 mM KCl, 20 mM HEPES pH

7.6, 1 mM MgCl$_2$, 1% glycerol, 0.5% NP-40, 50 mM beta-mercaptoethanol, 1 mg/ml

BSA, cocktail of protease inhibitors) along with a test agent to the wells of a Neutralite-

avidin coated assay plate and incubated at 25°C for 1 hour. Biotinylated substrate is then

added to each well and incubated for 1 hour. Reactions are stopped by washing with PBS,

25   and counted in a scintillation counter. Test agents that cause a difference in activity

relative to control without test agent are identified as candidate p53 modulating agents.

IV.   Immunoprecipitations and Immunoblotting

For coprecipitation of transfected proteins, 3×10^6 appropriate recombinant cells

30   containing the LCE proteins are plated on 10-cm dishes and transfected on the following

day with expression constructs. The total amount of DNA is kept constant in each

transfection by adding empty vector. After 24 h, cells are collected, washed once with

phosphate-buffered saline and lysed for 20 min on ice in 1 ml of lysis buffer containing 50

mM Hepes, pH 7.9, 250 mM NaCl, 20 mM -glycerophosphate, 1 mM sodium

orthovanadate, 5 mM p-nitrophenyl phosphate, 2 mM dithiothreitol, protease inhibitors (complete, Roche Molecular Biochemicals), and 1% Nonidet P-40. Cellular debris is removed by centrifugation twice at 15,000 × g for 15 min. The cell lysate is incubated with 25 µl of M2 beads (Sigma) for 2 h at 4 °C with gentle rocking.

5   After extensive washing with lysis buffer, proteins bound to the beads are solubilized by boiling in SDS sample buffer, fractionated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane and blotted with the indicated antibodies. The reactive bands are visualized with horseradish peroxidase coupled to the appropriate secondary antibodies and the enhanced chemiluminescence (ECL) Western

10   blotting detection system (Amersham Pharmacia Biotech).

V.   Expression analysis

All cell lines used in the following experiments are NCI (National Cancer Institute) lines, and are available from ATCC (American Type Culture Collection, Manassas, VA

15   20110-2209). Normal and tumor tissues were obtained from Impath, UC Davis, Clontech, Stratagene, and Ambion.

TaqMan analysis was used to assess expression levels of the disclosed genes in various samples.

RNA was extracted from each tissue sample using Qiagen (Valencia, CA) RNeasy

20   kits, following manufacturer's protocols, to a final concentration of 50ng/µl. Single stranded cDNA was then synthesized by reverse transcribing the RNA samples using random hexamers and 500ng of total RNA per reaction, following protocol 4304965 of Applied Biosystems (Foster City, CA, http://www.appliedbiosystems.com/).

Primers for expression analysis using TaqMan assay (Applied Biosystems, Foster City,

25   CA) were prepared according to the TaqMan protocols, and the following criteria: a) primer pairs were designed to span introns to eliminate genomic contamination, and b) each primer pair produced only one product.

Taqman reactions were carried out following manufacturer's protocols, in 25 µl total volume for 96-well plates and 10 µl total volume for 384-well plates, using 300nM primer

30   and 250 nM probe, and approximately 25ng of cDNA. The standard curve for result analysis was prepared using a universal pool of human cDNA samples, which is a mixture of cDNAs from a wide variety of tissues so that the chance that a target will be present in appreciable amounts is good. The raw data were normalized using 18S rRNA (universally expressed in all tissues and cells).

For each expression analysis, tumor tissue samples were compared with matched normal tissues from the same patient. A gene was considered overexpressed in a tumor when the level of expression of the gene was 2 fold or higher in the tumor compared with its matched normal sample. In cases where normal tissue was not available, a universal pool of cDNA samples was used instead. In these cases, a gene was considered overexpressed in a tumor sample when the difference of expression levels between a tumor sample and the average of all normal samples from the same tissue type was greater than 2 times the standard deviation of all normal samples (i.e., Tumor – average(all normal samples) > 2 x STDEV(all normal samples)).

Results are shown in Table 1. Data presented in bold indicate that greater than 50% of tested tumor samples of the tissue type indicated in row 1 exhibited over expression of the gene listed in column 1, relative to normal samples. Underlined data indicates that between 25% to 49% of tested tumor samples exhibited over expression. A modulator identified by an assay described herein can be further validated for therapeutic effect by administration to a tumor in which the gene is overexpressed. A decrease in tumor growth confirms therapeutic utility of the modulator. Prior to treating a patient with the modulator, the likelihood that the patient will respond to treatment can be diagnosed by obtaining a tumor sample from the patient, and assaying for expression of the gene targeted by the modulator. The expression data for the gene(s) can also be used as a diagnostic marker for disease progression. The assay can be performed by expression analysis as described above, by antibody directed to the gene target, or by any other available detection method.

Table 1

	breast		colon		lung			ovary	
GI# 10444344 (SEQ ID NO:1)	1	12	8	29	3	14		2	7
GI#10440044 (SEQ ID NO:4)	3	12	4	30	9	14		3	7

VI. Loss of function of the *Drosophila* CIG30/LCE gene is associated with tracheal defects

Genetic screens were designed to identify modifiers of branching morphogenesis in *Drosophila*. Briefly, *Drosophila* embryos (approximately stage 16) that were homozygous for lethal insertions of a *piggyBac* (Fraser M et al., Virology (1985) 145:356-361) or P-element transposon were screened for tracheal defects using monoclonal antibody 2A12

(Samakovlis C, et al., Development (1996) 122:1395-1407; Patel NH. (1994) Practical

Uses in Cell and Molecular Biology. Eds LSB Goldstein and EA Fryberg. Vol 44 pp446-

488. San Diego Academic Press). Sequence information surrounding the transposon

insertion site was used to identify the gene mutated by the insertion. The homozygous

5   disruption of the *Drosophila* CIG30 (*baldspot*) gene was identified as associated with

trachael defects.

VII.   Loss of function of a zebrafish LCEs is associated with branching morphogenesis
        defects

10   Using antisense technologies, we identified vasculature defects associated with loss-

of-function of the zebrafish (Danio rerio) LCE that we have designated DrCIG30L4,

whose nucleic acid and polypeptide sequences are presented, respectively, in SEQ ID

NO:7and SEQ ID NO:15. We have identified ten candidate zebrafish LCE genes. Of

these, four were tested for involvement in angiogenesis using essentially the following

15   methods. Wild type, one-cell stage embryos from the Tübingen strain were treated with

antisense morpholino oligonucleotide (PMOs) that targeted predicted zebrafish genes.

PMOs were dissolved in injection buffer (0.4 mM MgSO$_4$, 0.6 mM CaCl$_2$, 0,7 mM KCl,

58 mM NaCl, 25 mM Hepes [pH 7,6]), and 2-8 ng was injected into zebrafish embryos at

the 1-cell stage.

20   Larvae were fixed at 4 days post fertilization (dpf) in 4% para-formaldehyde in

phosphate-buffered saline (PBS) for 30 minutes. Fixed larvae were dehydrated in

methanol and stored over night at -20°C. After permeabilization in acetone (30 minutes at

-20°C), embryos were washed in PBS and incubated in the staining buffer (100 mM Tris-

HCl [pH 9.5], 50mM MgCl$_2$, 100mM NaCl, 0.1% Tween-20) for 45 minutes. Staining

25   reaction was started by adding 2.25 µl nitro blue tetrazolium (NBT, Sigma) and 1.75 µl 5-

bromo-4-chloro-3-indolyl phosphate (BCIP, Sigma) per ml of staining buffer (stock

solutions: 75 mg/ml NBT in 70% N,N-dimethylformamide, 50 mg/ml BCIP in N,N-

dimethylformamide).

The fixed specimens were scanned for changes in blood vessel formation. Treatment

30   of embryos with a PMO corresponding to the complement of nucleotides 692-712 of SEQ

ID NO:7 (nucleotide sequence), produced a dose-dependant block of vasculogenesis and

angiogenesis. Following injection of 2ng of the PMO, the intersegmental vessels (ISV)

were disrupted; at 4ng of the PMO, the loss of ISV was much more severe. Following

injection of 8ng of PMO, the loss of blood vessels is more severe with few ISV and the subintestinal vein (SIV) was almost complete disrupted.

VIII. *ELOVL4* is an angiogenesis gene

5   Based on analysis of the zebrafish DrCIG30L4, we have identified *ELOVL4* as an angiogenesis gene. We used computation analysis, specifically BLAST, Smith-Waterman, and CLUSTALW analysis, to identify human *ELOVL4* ("HsELOVL4," GI#s 12044043 and 12232379, SEQ ID NOs:13 and 14) as the ortholog of DrCIG30L4. The Drosophila CIG30 protein sequence was BLASTed against a *Homo sapiens* amino acid

10   sequence database to identify the family of CIG30 and ELOV (fatty acid elongases). The *Homo sapiens* sequences were similarly used to BLAST analyze the available zebrafish amino acid sequences both from public databases as well as our internal sequence databases. Initially, four zebrafish homologs to the CIG30/ELOV family were identified. To reassess the orthology of the DrCIG30L4 and other zebrafish orthologs, these were

15   BLAST analyzed individually against the *Homo sapiens* amino acid sequence database. In the case of DrCIG30L4, the top "hit" was *Homo sapiens* ELOVL4. *Homo sapiens* ELOVL4 amino acid sequence was then used to BLAST against the zebrafish amino acid sequence database; the DrCIG30L4 sequence was the mutual best "hit". ClustalW alignment and phylogenetic analysis of the zebrafish CIG30/ELOV family also showed

20   that HsELOVL4 and DrCIG30L4 are most closely related.

Without intent to be limiting, we have contemplated links between *ELOVL4* and branching morphogenesis. As described above, *ELOVL4* is associated with Autosomal Dominant Stargardt-like Macular Dystrophy (STDG3). While a direct link between STGD3, *ELOVL4* and angiogenesis has not been made, the PMO results in zebrafish

25   suggest a possible causal link between a defect in blood vessel formation and the phenotypes associated with STGD3.

Thus, we have identified ELOVE4 as an attractive drug target for the treatment of pathologies associated with branching morphogenesis, such as angiogenesis, particularly pathologies that are also associated with defective p53 signaling.

30

WHAT IS CLAIMED IS:

1.   A method of identifying a candidate p53 pathway modulating agent, said method comprising the steps of:

5   (a) providing an assay system comprising a purified LCE polypeptide or nucleic acid or a functionally active fragment or derivative thereof;

(b) contacting the assay system with a test agent under conditions whereby, but for the presence of the test agent, the system provides a reference activity; and

(c) detecting a test agent-biased activity of the assay system, wherein a difference

10   between the test agent-biased activity and the reference activity identifies the test agent as a candidate p53 pathway modulating agent.

2.   The method of Claim 1 wherein the assay system comprises cultured cells that express the LCE polypeptide.

15

3.   The method of Claim 2 wherein the cultured cells additionally have defective p53 function.

4.   The method of Claim 1 wherein the assay system includes a screening assay

20   comprising a LCE polypeptide, and the candidate test agent is a small molecule modulator.

5.   The method of Claim 4 wherein the assay is a binding assay.

6.   The method of Claim 1 wherein the assay system is selected from the group

25   consisting of an apoptosis assay system, a cell proliferation assay system, an angiogenesis assay system, and a hypoxic induction assay system.

7.   The method of Claim 1 wherein the assay system includes a binding assay comprising a LCE polypeptide and the candidate test agent is an antibody.

30

8.   The method of Claim 1 wherein the assay system includes an expression assay comprising a LCE nucleic acid and the candidate test agent is a nucleic acid modulator.

9.   The method of claim 8 wherein the nucleic acid modulator is an antisense oligomer.

10.   The method of Claim 8 wherein the nucleic acid modulator is a PMO.

5

11.   The method of Claim 1 additionally comprising:

(d) administering the candidate p53 pathway modulating agent identified in (c) to a model system comprising cells defective in p53 function and, detecting a phenotypic change in the model system that indicates that the p53 function is restored.

10

12.   The method of Claim 11 wherein the model system is a mouse model with defective p53 function.

13.   A method for modulating a p53 pathway of a cell comprising contacting a cell

15   defective in p53 function with a candidate modulator that specifically binds to a LCE polypeptide comprising an amino acid sequence selected from group consisting of SEQ ID NOs:9, 10, 11, 12, 13, 14, 15, and 16, whereby p53 function is restored.

14.   The method of claim 13 wherein the candidate modulator is administered to a

20   vertebrate animal predetermined to have a disease or disorder resulting from a defect in p53 function.

15.   The method of Claim 13 wherein the candidate modulator is selected from the group consisting of an antibody and a small molecule.

25

16.   The method of Claim 1, comprising the additional steps of:

(d) providing a secondary assay system comprising cultured cells or a non-human animal expressing LCE ,

(e) contacting the secondary assay system with the test agent of (b) or an agent

30   derived therefrom under conditions whereby, but for the presence of the test agent or agent derived therefrom, the system provides a reference activity; and

(f) detecting an agent-biased activity of the second assay system,

wherein a difference between the agent-biased activity and the reference activity of the second assay system confirms the test agent or agent derived therefrom as a candidate p53 pathway modulating agent,

and wherein the second assay detects an agent-biased change in the p53 pathway.

5

17.   The method of Claim 16 wherein the secondary assay system comprises cultured cells.

18.   The method of Claim 16 wherein the secondary assay system comprises a non-

10   human animal.

19.   The method of Claim 18 wherein the non-human animal mis-expresses a p53 pathway gene.

15   20.   A method of modulating p53 pathway in a mammalian cell comprising contacting the cell with an agent that specifically binds a LCE polypeptide or nucleic acid.

21.   The method of Claim 20 wherein the agent is administered to a mammalian animal predetermined to have a pathology associated with the p53 pathway.

20

22.   The method of Claim 20 wherein the agent is a small molecule modulator, a nucleic acid modulator, or an antibody.

23.   A method for diagnosing a disease in a patient comprising:

25   (a) obtaining a biological sample from the patient;

           (b) contacting the sample with a probe for LCE expression;

           (c) comparing results from step (b) with a control;

           (d) determining whether step (c) indicates a likelihood of disease.

30   24.   The method of claim 23 wherein said disease is cancer.

25.   The method according to claim 24, wherein said cancer is a cancer as shown in Table 1 as having >25% expression level.

26.   A method of identifying a candidate branching morphogenesis modulating agent, said method comprising the steps of:

(a) providing an assay system comprising an ELOVL4 polypeptide or nucleic acid;

(b) contacting the assay system with a test agent under conditions whereby, but for

5   the presence of the test agent, the system provides a reference activity; and

(c) detecting a test agent-biased activity of the assay system, wherein a difference between the test agent-biased activity and the reference activity identifies the test agent as a candidate branching morphogenesis modulating agent.

10   27.   The method of Claim 26 wherein the assay system comprises cultured cells or a non-human animal expressing ELOVL4,
and wherein the assay system includes an assay that detects an agent-biased change in branching morphogenesis.

15   28.   The method of Claim 27 wherein the branching morphogenesis is angiogenesis.

29.   The method of Claim 27 wherein the assay system comprises cultured cells and wherein the assay detects an event selected from the group consisting of cell proliferation, cell cycling, apoptosis, tubulogenesis, cell migration, cell sprouting and response to

20   hypoxic conditions.

30.   The method of Claim 27 wherein the assay system comprises a non-human animal and wherein the assay system includes a matrix implant (Matrigel) assay or a xenograft assay.

25

31.   The method of Claim 26, comprising the additional steps of:

(d) providing a second assay system comprising cultured cells or a non-human animal expressing ELOVL4,

(e) contacting the second assay system with the test agent of (b) or an agent

30   derived therefrom under conditions whereby, but for the presence of the test agent or agent derived therefrom, the system provides a reference activity; and

(f) detecting an agent-biased activity of the second assay system,

wherein a difference between the agent-biased activity and the reference activity of the

second assay system confirms the test agent or agent derived therefrom as a candidate

branching morphogenesis modulating agent,

and wherein the second assay system includes a second assay that detects an agent-biased

5   change in an activity associated with branching morphogenesis.

32.   A method of modulating branching morphogenesis in a mammalian cell

comprising contacting the cell with an agent that specifically binds an ELOVL4

polypeptide or nucleic acid.

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<213> Homo sapiens

<400> 4
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7

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8

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12

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13

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Leu Ile Leu Trp Ser Phe Cys Leu Ala Ile Phe Ser Ile Leu Gly Ala
            35                  40                  45


Val Arg Met Trp Gly Ile Met Gly Thr Val Leu Leu Thr Gly Gly Leu
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Lys Gln Thr Val Cys Phe Ile Asn Phe Ile Asp Asn Ser Thr Val Lys
65                  70                  75                  80
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14

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Phe Trp Ser Trp Val Phe Leu Leu Ser Lys Val Ile Glu Leu Gly Asp
                85                  90                  95


Thr Ala Phe Ile Ile Leu Arg Lys Arg Pro Leu Ile Phe Ile His Trp
            100                 105                 110


Tyr His His Ser Thr Val Leu Val Tyr Thr Ser Phe Gly Tyr Lys Asn
            115                 120                 125


Lys Val Pro Ala Gly Gly Trp Phe Val Thr Met Asn Phe Gly Val His
    130                 135                 140


Ala Ile Met Tyr Thr Tyr Tyr Thr Leu Lys Ala Ala Asn Val Lys Pro
145                 150                 155                 160


Pro Lys Met Leu Pro Met Leu Ile Thr Ser Leu Gln Ile Leu Gln Met
                165                 170                 175


Phe Val Gly Ala Ile Val Ser Ile Leu Thr Tyr Ile Trp Arg Gln Asp
                180                 185                 190


Gln Gly Cys His Thr Thr Met Glu His Leu Phe Trp Ser Phe Ile Leu
            195                 200                 205


Tyr Met Thr Tyr Phe Ile Leu Phe Ala His Phe Phe Cys Gln Thr Tyr
    210                 215                 220


Ile Arg Pro Lys Val Lys Ala Lys Thr Lys Ser Gln
225                 230                 235
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1               5               10                  15


Pro Tyr Asn Phe Glu Leu Ser Lys Asp Met Arg Pro Phe Phe Glu Glu
            20                  25                  30


Tyr Trp Ala Thr Ser Phe Pro Ile Ala Leu Ile Tyr Leu Val Leu Ile
            35                  40                  45


Ala Val Gly Gln Asn Tyr Met Lys Glu Arg Lys Gly Phe Asn Leu Gln
    50                  55                  60
```

15

Gly Pro Leu Ile Leu Trp Ser Phe Cys Leu Ala Ile Phe Ser Ile Leu
65   70   75   80

Gly Ala Val Arg Met Trp Gly Ile Met Gly Thr Val Leu Leu Thr Gly
               85   90   95

Gly Leu Lys Gln Thr Val Cys Phe Ile Asn Phe Ile Asp Asn Ser Thr
         100   105   110

Val Lys Phe Trp Ser Trp Val Phe Leu Leu Ser Lys Val Ile Glu Leu
         115   120   125

Gly Asp Thr Ala Phe Ile Ile Leu Arg Lys Arg Pro Leu Ile Phe Ile
    130   135   140

His Trp Tyr His His Ser Thr Val Leu Val Tyr Thr Ser Phe Gly Tyr
145   150   155   160

Lys Asn Lys Val Pro Ala Gly Gly Trp Phe Val Thr Met Asn Phe Gly
              165   170   175

Val His Ala Ile Met Tyr Thr Tyr Tyr Thr Leu Lys Ala Ala Asn Val
              180   185   190

Lys Pro Pro Lys Met Leu Pro Met Leu Ile Thr Ser Leu Gln Ile Leu
         195   200   205

Gln Met Phe Val Gly Ala Ile Val Ser Ile Leu Thr Tyr Ile Trp Arg
    210   215   220

Gln Asp Gln Gly Cys His Thr Thr Met Glu His Leu Phe Trp Ser Phe
225   230   235   240

Ile Leu Tyr Met Thr Tyr Phe Ile Leu Phe Ala His Phe Phe Cys Gln
              245   250   255

Thr Tyr Ile Arg Pro Lys Val Lys Ala Lys Thr Lys Ser Gln
              260   265   270

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<400>   11

Met Asn Met Ser Val Leu Thr Leu Gln Glu Tyr Glu Phe Glu Lys Gln

16

1   5   10   15

Phe Asn Glu Asn Glu Ala Ile Gln Trp Met Gln Glu Asn Trp Lys Lys
            20   25   30

Ser Phe Leu Phe Ser Ala Leu Tyr Ala Ala Phe Ile Phe Gly Gly Arg
            35   40   45

His Leu Met Asn Lys Arg Ala Lys Phe Glu Leu Arg Lys Pro Leu Val
            50   55   60

Leu Trp Ser Leu Thr Leu Ala Val Phe Ser Ile Phe Gly Ala Leu Arg
65   70   75   80

Thr Gly Ala Tyr Met Val Tyr Ile Leu Met Thr Lys Gly Leu Lys Gln
                  85   90   95

Ser Val Cys Asp Gln Gly Phe Tyr Asn Gly Pro Val Ser Lys Phe Trp
            100   105   110

Ala Tyr Ala Phe Val Leu Ser Lys Ala Pro Glu Leu Gly Asp Thr Ile
            115   120   125

Phe Ile Ile Leu Arg Lys Gln Lys Leu Ile Phe Leu His Trp Tyr His
            130   135   140

His Ile Thr Val Leu Leu Tyr Ser Trp Tyr Ser Tyr Lys Asp Met Val
145   150   155   160

Ala Gly Gly Gly Trp Phe Met Thr Met Asn Tyr Gly Val His Ala Val
                  165   170   175

Met Tyr Ser Tyr Tyr Ala Leu Arg Ala Ala Gly Phe Arg Val Ser Arg
            180   185   190

Lys Phe Ala Met Phe Ile Thr Leu Ser Gln Ile Thr Gln Met Leu Met
            195   200   205

Gly Cys Val Val Asn Tyr Leu Val Phe Cys Trp Met Gln His Asp Gln
            210   215   220

Cys His Ser His Phe Gln Asn Ile Phe Trp Ser Ser Leu Met Tyr Leu
225   230   235   240

Ser Tyr Leu Val Leu Phe Cys His Phe Phe Phe Glu Ala Tyr Ile Gly
                  245   250   255

17

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Lys Met Arg Lys Thr Thr Lys Ala Glu
            260                 265


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<213>  Homo sapiens


<400>  12

Met Asn Met Ser Val Leu Thr Leu Gln Glu Tyr Glu Phe Glu Lys Gln
1               5                   10                  15


Phe Asn Glu Asn Glu Ala Ile Gln Trp Met Gln Glu Asn Trp Lys Lys
            20                  25                  30


Ser Phe Leu Phe Ser Ala Leu Tyr Ala Ala Phe Ile Phe Gly Gly Arg
        35                  40                  45


His Leu Met Asn Lys Arg Ala Lys Phe Glu Leu Arg Lys Pro Leu Val
    50                  55                  60


Leu Trp Ser Leu Thr Leu Ala Val Phe Ser Ile Phe Gly Ala Leu Arg
65              70                  75                  80


Thr Gly Ala Tyr Met Val Tyr Ile Leu Met Thr Lys Gly Leu Lys Gln
                85                  90                  95


Ser Val Cys Asp Gln Gly Phe Tyr Asn Gly Pro Val Ser Lys Phe Trp
            100                 105                 110


Ala Tyr Ala Phe Val Leu Ser Lys Ala Pro Glu Leu Gly Asp Thr Ile
        115                 120                 125


Phe Ile Ile Leu Arg Lys Gln Lys Leu Ile Phe Leu His Trp Tyr His
    130                 135                 140


His Ile Thr Val Leu Leu Tyr Ser Trp Tyr Ser Tyr Lys Asp Met Val
145                 150                 155                 160


Ala Gly Gly Gly Trp Phe Met Thr Met Asn Tyr Gly Val His Ala Val
                165                 170                 175


Met Tyr Ser Tyr Tyr Ala Leu Arg Ala Ala Gly Phe Arg Val Ser Arg
            180                 185                 190


Lys Phe Ala Met Phe Ile Thr Leu Ser Gln Ile Thr Gln Met Leu Met
        195                 200                 205
```

Gly Cys Val Val Asn Tyr Leu Val Phe Cys Trp Met Gln His Asp Gln
    210   215   220

Cys His Ser His Phe Gln Asn Ile Phe Trp Ser Ser Leu Met Tyr Leu
225   230   235   240

Ser Tyr Leu Val Leu Phe Cys His Phe Phe Glu Ala Tyr Ile Gly
            245   250   .   255

Lys Met Arg Lys Thr Thr Lys Ala Glu
            260   265

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<212> PRT
<213> Homo sapiens

<400> 13

Met Gly Leu Leu Asp Ser Glu Pro Gly Ser Val Leu Asn Val Val Ser
1   5   10   15

Thr Ala Leu Asn Asp Thr Val Glu Phe Tyr Arg Trp Thr Trp Ser Ile
            20   25   30

Ala Asp Lys Arg Val Glu Asn Trp Pro Leu Met Gln Ser Pro Trp Pro
        35   40   45

Thr Leu Ser Ile Ser Thr Leu Tyr Leu Leu Phe Val Trp Leu Gly Pro
    50   55   60

Lys Trp Met Lys Asp Arg Glu Pro Phe Gln Met Arg Leu Val Leu Ile
65   70   75   80

Ile Tyr Asn Phe Gly Met Val Leu Leu Asn Leu Phe Ile Phe Arg Glu
            85   90   95

Leu Phe Met Gly Ser Tyr Asn Ala Gly Tyr Ser Tyr Ile Cys Gln Ser
            100   105   110

Val Asp Tyr Ser Asn Asn Val His Glu Val Arg Ile Ala Ala Ala Leu
        115   120   125

Trp Trp Tyr Phe Val Ser Lys Gly Val Glu Tyr Leu Asp Thr Val Phe
    130   135   140

Phe Ile Leu Arg Lys Lys Asn Asn Gln Val Ser Phe Leu His Val Tyr

19

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        145                    150                  155                    160


        His His Cys Thr Met Phe Thr Leu Trp Trp Ile Gly Ile Lys Trp Val
                        165                  170                    175


        Ala Gly Gly Gln Ala Phe Phe Gly Ala Gln Leu Asn Ser Phe Ile His
                        180                  185                    190


        Val Ile Met Tyr Ser Tyr Tyr Gly Leu Thr Ala Phe Gly Pro Trp Ile
                        195                  200                    205


        Gln Lys Tyr Leu Trp Trp Lys Arg Tyr Leu Thr Met Leu Gln Leu Ile
                        210                  215                    220


        Gln Phe His Val Thr Ile Gly His Thr Ala Leu Ser Leu Tyr Thr Asp
        225                      230                  235                    240


        Cys Pro Phe Pro Lys Trp Met His Trp Ala Leu Ile Ala Tyr Ala Ile
                        245                  250                    255


        Ser Phe Ile Phe Leu Phe Leu Asn Phe Tyr Ile Arg Thr Tyr Lys Glu
                        260                  265                    270


        Pro Lys Lys Pro Lys Ala Gly Lys Thr Ala Met Asn Gly Ile Ser Ala
                        275                  280                    285


        Asn Gly Val Ser Lys Ser Glu Lys Gln Leu Met Ile Glu Asn Gly Lys
                290                  295                  300


        Lys Gln Lys Asn Gly Lys Ala Lys Gly Asp
        305                      310
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<400> 14

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Met Gly Leu Leu Asp Ser Glu Pro Gly Ser Val Leu Asn Val Val Ser
1                   5                   10                    15


Thr Ala Leu Asn Asp Thr Val Glu Phe Tyr Arg Trp Thr Trp Ser Ile
                20                  25                    30


Ala Asp Lys Arg Val Glu Asn Trp Pro Leu Met Gln Ser Pro Trp Pro
                35                  40                    45
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Thr Leu Ser Ile Ser Thr Leu Tyr Leu Leu Phe Val Trp Leu Gly Pro
    50              55              60

Lys Trp Met Lys Asp Arg Glu Pro Phe Gln Met Arg Leu Val Leu Ile
65              70              75              80

Ile Tyr Asn Phe Gly Met Val Leu Leu Asn Leu Phe Ile Phe Arg Glu
            85              90              95

Leu Phe Met Gly Ser Tyr Asn Ala Gly Tyr Ser Tyr Ile Cys Gln Ser
            100             105             110

Val Asp Tyr Ser Asn Asn Val His Glu Val Arg Ile Ala Ala Ala Leu
        115             120             125

Trp Trp Tyr Phe Val Ser Lys Gly Val Glu Tyr Leu Asp Thr Val Phe
    130             135             140

Phe Ile Leu Arg Lys Lys Asn Asn Gln Val Ser Phe Leu His Val Tyr
145             150             155             160

His His Cys Thr Met Phe Thr Leu Trp Trp Ile Gly Ile Lys Trp Val
            165             170             175

Ala Gly Gly Gln Ala Phe Phe Gly Ala Gln Leu Asn Ser Phe Ile His
            180             185             190

Val Ile Met Tyr Ser Tyr Tyr Gly Leu Thr Ala Phe Gly Pro Trp Ile
        195             200             205

Gln Lys Tyr Leu Trp Trp Lys Arg Tyr Leu Thr Met Leu Gln Leu Ile
    210             215             220

Gln Phe His Val Thr Ile Gly His Thr Ala Leu Ser Leu Tyr Thr Asp
225             230             235             240

Cys Pro Phe Pro Lys Trp Met His Trp Ala Leu Ile Ala Tyr Ala Ile
            245             250             255

Ser Phe Ile Phe Leu Phe Leu Asn Phe Tyr Ile Arg Thr Tyr Lys Glu
            260             265             270

Pro Lys Lys Pro Lys Ala Gly Lys Thr Ala Met Asn Gly Ile Ser Ala
        275             280             285

Asn Gly Val Ser Lys Ser Glu Lys Gln Leu Met Ile Glu Asn Gly Lys
290             295             300
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21

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<210> 15
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1   5   10   15

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            20   25   30

Ser Ser Pro Leu Pro Thr Leu Gly Ile Ser Val Leu Tyr Leu Leu Phe
            35   40   45

Leu Trp Ala Gly Pro Leu Tyr Met Gln Asn Arg Glu Pro Phe Gln Leu
        50   55   60

Arg Lys Thr Leu Ile Val Tyr Asn Phe Ser Met Val Leu Leu Asn Phe
65   70   75   80

Tyr Ile Cys Lys Glu Leu Leu Leu Gly Ser Arg Ala Ala Gly Tyr Ser
                85   90   95

Tyr Leu Cys Gln Pro Val Asn Tyr Ser Asn Asp Val Asn Glu Val Arg
                100   105   110

Ile Ala Ser Ala Leu Trp Trp Tyr Tyr Ile Ser Lys Gly Val Glu Phe
            115   120   125

Leu Asp Thr Val Phe Phe Ile Met Arg Lys Lys Phe Asn Gln Val Ser
            130   135   140

Phe Leu His Val Tyr His His Cys Thr Met Phe Ile Leu Trp Trp Ile
145   150   155   160

Gly Ile Lys Trp Val Pro Gly Gly Gln Ser Phe Phe Gly Ala Thr Ile
                165   170   175

Asn Ser Gly Ile His Val Leu Met Tyr Gly Tyr Tyr Gly Leu Ala Ala
                180   185   190

Phe Gly Pro Lys Ile Gln Lys Tyr Leu Trp Trp Lys Lys Tyr Leu Thr

22

                195   200   205

Ile Ile Gln Met Ile Gln Phe His Val Thr Ile Val His Ala Ala Tyr
    210   215   220

Ser Leu Tyr Thr Gly Cys Pro Phe Pro Ala Trp Met Gln Ser Ala Leu
225   230   235   240

Ile Gly Tyr Ala Asp Thr Phe Ile Ile Leu Leu Ala Asn Phe Tyr Tyr
            245   250   255

Gln Thr Tyr Arg Arg Gln Pro Leu Pro Lys Thr Ala Lys Phe Ala Val
        260   265   270

Asn Gly Val Ser Met Ser Thr Asn Gly Thr Ser Lys Thr Ala Glu Val
        275   280   285

Thr Glu Asn Gly Lys Lys Gln Thr Lys Gly Lys Gly Lys His Asp
    290   295   300

<210>   16
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<212>   PRT
<213>   Homo sapiens

<400>   16

Met Val Thr Ala Met Asn Val Ser His Glu Val Asn Gln Leu Phe Gln
1   5   10   15

Pro Tyr Asn Phe Glu Leu Ser Lys Asp Met Arg Pro Phe Phe Glu Glu
            20   25   30

Tyr Trp Ala Thr Ser Phe Pro Ile Ala Leu Ile Tyr Leu Val Leu Ile
        35   40   45

Ala Val Gly Gln Asn Tyr Met Lys Glu Arg Lys Gly Phe Asn Leu Gln
    50   55   60

Gly Pro Leu Ile Leu Trp Ser Phe Cys Leu Ala Ile Phe Ser Ile Leu
65   70   75   80

Gly Ala Val Arg Met Trp Gly Ile Met Gly Thr Val Leu Leu Thr Gly
            85   90   95

Gly Leu Lys Gln Thr Val Cys Phe Ile Asn Phe Ile Asp Asn Ser Thr
        100   105   110

Val Lys Phe Trp Ser Trp Val Phe Leu Leu Ser Lys Val Ile Glu Leu
        115   120   125

Gly Asp Thr Ala Phe Ile Ile Leu Arg Lys Arg Pro Leu Ile Phe Ile
        130   135   140

His Trp Tyr His His Ser Thr Val Leu Val Tyr Thr Ser Phe Gly Tyr
145   150   155   160

Lys Asn Lys Val Pro Ala Gly Gly Trp Phe Val Thr Met Asn Phe Gly
                165   170   175

Val His Ala Ile Met Tyr Thr Tyr Tyr Thr Leu Lys Ala Ala Asn Val
            180   185   190

Lys Pro Pro Lys Met Leu Pro Met Leu Ile Thr Ser Leu Gln Ile Leu
        195   200   205

Gln Met Phe Val Gly Ala Ile Val Ser Ile Leu Thr Tyr Ile Trp Arg
        210   215   220

Gln Asp Gln Gly Cys His Thr Thr Met Glu His Leu Phe Trp Ser Phe
225   230   235   240

Ile Leu Tyr Met Thr Tyr Phe Ile Leu Phe Ala His Phe Phe Cys Gln
                245   250   255

Thr Tyr Ile Arg Pro Lys Val Lys Ala Lys Thr Lys Ser Gln
            260   265   270

(51) International Patent Classification[7]:   C12Q 1/00,
G01N 33/567, 33/53, C12P 21/06

(21) International Application Number: PCT/US02/17739

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(25) Filing Language: English

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(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
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(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR,
GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent
(BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR,
NE, SN, TD, TG).

Published:
— with international search report

(88) Date of publication of the international search report:
4 December 2003

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: LCES AS MODIFIERS OF THE p53 PATHWAY AND METHODS OF USE

(57) Abstract: Human LCE genes are identified as modulators of the p53 pathway, and thus are therapeutic targets for disorders
associated with defective p53 function. Methods for identifying modulators of p53, comprising screening for agents that modulate
the activity of LCE are provided.

INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER
IPC(7)	: C12Q 1/00; G01N 33/567, 33/53; C12P 21/06
US CL	: 435/4, 7.21, 7.91, 68.1

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 435/4, 7.21, 7.91, 68.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C.	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOON et al. Identification of a mammalian long chain fatty acyl elongase regulated by sterol regulatory element-binding proteins. Journal of Biological Chemistry. 2001, Vol. 276, No. 48, pages 45358-45366. See entire document.	1-3 (in part), 4-5, 6 (in part), 7, 11-12 and 16-19
Y	WO 02/07586 A2 (THE UNIVERSITY OF BRITISH COLUMBIA) 01 February 2001 (01.02.2001), see entire document.	1-3 (in part), 4-5, 6 (in part), 7, 11-12 and 16-19
T/A	WO 02/062975 A2 (BAYER AKTIENGESELLSCHAFT) 15 August 2002 (15.08.2002), see entire document.	1-3 (in part), 4-5, 6 (in part), 7, 11-12 and 16-19
Y	WO 00/055330 A1 (THE UNIVERSITY OF BRISTOL) 21 September 2000 (21.09.2000), see entire document.	1-3 (in part), 4-5, 6 (in part), 7, 11-12 and 16-19

☐ Further documents are listed in the continuation of Box C.   ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier application or patent published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 March 2003 (19.03.2003)	1 4 MAY 2003
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Robert A. Zeman
Facsimile No. (703)305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/17739

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-3 (in part), 4-5, 6 (in part), 7, 11-12 and 16-19

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-3.(in part), 4-5, 6 (in part), 7, 11-12, and 16-19, drawn to methods of identifying a candidate p53 pathway modulating agent using a purified LCE polypeptide.

Group II, claim(s) 1-3 (in part), 6 (in part), and 8-10, drawn to methods of identifying a candidate p53 pathway modulating agent using a purified LCE polynucleotide.

Group III, claim(s) 13-15 (in part) and 20-22 (in part), drawn to methods of modulating a p53 pathway comprising contacting a cell with a modulator that specifically binds an LCE polypeptide comprising the amino acid sequence of SEQ ID NO:9.

Group IV, claim(s) 13-15 (in part) and 20-22 (in part), drawn to methods of modulating a p53 pathway comprising contacting a cell with a modulator that specifically binds an LCE polypeptide comprising the amino acid sequence of SEQ ID NO:10.

Group V, claim(s) 13-15 (in part) and 20-22 (in part), drawn to methods of modulating a p53 pathway comprising contacting a cell with a modulator that specifically binds an LCE polypeptide comprising the amino acid sequence of SEQ ID NO:11.

Group VI, claim(s) 13-15 (in part) and 20-22 (in part), drawn to methods of modulating a p53 pathway comprising contacting a cell with a modulator that specifically binds an LCE polypeptide comprising the amino acid sequence of SEQ ID NO:12.

Group VII, claim(s) 13-15 (in part) and 20-22 (in part), drawn to methods of modulating a p53 pathway comprising contacting a cell with a modulator that specifically binds an LCE polypeptide comprising the amino acid sequence of SEQ ID NO:13.

Group VIII, claim(s) 13-15 (in part) and 20-22 (in part), drawn to methods of modulating a p53 pathway comprising contacting a cell with a modulator that specifically binds an LCE polypeptide comprising the amino acid sequence of SEQ ID NO:14.

Group IX, claim(s) 13-15 (in part) and 20-22 (in part), drawn to methods of modulating a p53 pathway comprising contacting a cell with a modulator that specifically binds an LCE polypeptide comprising the amino acid sequence of SEQ ID NO:15.

Group X, claim(s) 13-15 (in part) and 20-22 (in part), drawn to methods of modulating a p53 pathway comprising contacting a cell with a modulator that specifically binds an LCE polypeptide comprising the amino acid sequence of SEQ ID NO:16.

Group XI, claim(s) 20-22 (in part), drawn to methods of modulating a p53 pathway comprising contacting a cell with a modulator that specifically binds an LCE polynucleotide.

Group XII, claim(s) 23-25, drawn to methods for diagnosing a disease with a probe for LCE expression.

Group XIII, claim(s) 26-32, drawn to methods of identifying a candidate branching morphogenesis modulating agent with an ELOVL4 polypeptide.

The inventions listed as Groups I-XIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

An international and a national stage application shall relate to one invention only or to a group of inventions so linked as to form a single general inventive concept ("requirement of unity of invention"). Where a group of inventions is claimed in an application, the requirement of unity of invention shall be fulfilled only when there is a technical relationship among those inventions

involving one or more of the same or corresponding special technical features. An international or a national stage application containing claims to different categories of invention will be considered to have unity of invention if the claims are drawn only to one of the following combinations of categories: (1) a product and a process specially adapted for the manufacture of said product; or (2) a product and a process of use of said product; or (3) a product, a process specially adapted for the manufacture of the said product, and a use of the said product; or (4) a process and an apparatus or means specifically designed for carrying out the said process; or (5) a product, a process specially adapted for the manufacture of the said product, and an apparatus or means specifically designed for carrying out the said process. If multiple products, processes of manufacture or uses are claimed, the first invention of the category first mentioned in the claims of the application and the first recited invention of each of the other categories related thereto will be considered as the main invention in the claims, see PCT Article 17(3)(a) and 1.476(c).

In the present case, the claims of Group I share the special technical feature of methods of use of an LCE polypeptide for identifying a candidate p53 modulator. The claims of Group II are drawn to identification of a candidate p53 modulator with an LCE polynucleotide, which is a different technical feature than the method of use of the LCE polypeptide of Group I. The claims of Groups III-XI do not share the special technical feature of methods of identifying a candidate p53 modulator with an LCE polypeptide, but rather are drawn to the technical features of modulating a p53 pathway of a cell by contacting the cell with a modulator that binds one of the specifically recited polypeptides of the groups or with a modulator that binds an LCE nucleic acid. Therefore, these groups do not share unity of invention with Group I. Finally, the methods of Groups XII-XIII are drawn to methods of use of products other than an LCE polypeptide and thus do not share unity of invention with Group I.

Continuation of B. FIELDS SEARCHED Item 3:
Medline, Caplus, Biosis, USpatfull, Europatfull

p53, LCE, elongase, modulating, cells, apoptosis, cells, expression, binding assay, antibody, defective p53